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STUDIES ON CURLY-TOP DISEASE OF THE SUGAR BEET [1]

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INTRODUCTION

Curly-top [2] of sugar beets presumably was widespread before it was recognized as a distinct disease. It appears to have been confused at first with other diseases or other types of injury. The extensive losses in California, in 1899, referred to as the "California beet disease," "Western blight," etc., led to its being recognized as a distinct and serious disease, and the following year a similar condition was determined as occurring in all of the Western States where sugar beets were grown.

Linhart (*9*) [3] summarized the reports of several specialists with reference especially to California conditions. This report recorded the conditions of the areas infected but did not establish the cause or exact character of the injury.

Townsend (*19*) described and figured curly-top.

Ball (*1*) in the Annual Report for 1905 of the Utah Station announced the discovery of a causal relation between the feeding of the beet leafhopper and "curly-leaf" or "blight."

Smith (*14*) reported the severe outbreak of 1905 in California, stating that the injury was apparently physiological in character and possibly of the same general type of disease as tobacco mosaic.

Ball (*2*) emphasized, by further observation, the fact that "curly-leaf" is the result of leafhopper attack. He suggested that the disease itself might be in the nature of gall formation.

Wilhelmi (*21*) reported alkali injury as a possible cause of curly-top.

Townsend (*20*) described curly-top from both the pathological and geographical standpoints; while reaching the conclusion that the disease was a distinct systemic disease, he concluded that as yet no satisfactory explanation of the cause had been found.

Ball (*3*) adopted the name "curly-leaf" for the disease, reviewed the earlier work, and reported in considerable detail the relation of the sugar beet leafhopper and other leafhoppers to this and other injuries to the host plants. He emphasized especially that the curly-leaf condition, though produced by the leafhopper, is not due to mechanical injury by the insect nor to loss of sap. He pointed out that, in the case of injury produced in plants by related species of leafhoppers, the injury was apparently local in character; while in the case of the curly-leaf, the abnormal condition apparently spread from leaf to leaf, infecting the whole plant even though the leafhopper might have disappeared in the meantime. He also reported that the agency that caused curly-leaf was capable of remaining in the beet over winter, again producing the typical curly-leaf symptoms in the first leaves in the spring. He referred to the experimental production of "curly-leaf" in cages arranged jointly by himself and Prof. E. G.

[1] Received for publication Jan. 23, 1924.

[2] The name "curly-top" has been used in earlier publications of this Department. The question of the advisability of employing "curly-leaf" instead of "curly-top" as the common name of this disease has been referred to the American Phytopathological Society.

[3] Reference is made by number (italic) to "Literature cited," p. 318-319.

Titus, by H. B. Shaw and by the Office of Sugar-Beet Investigations, Bureau of Plant Industry, United States Department of Agriculture.

Shaw (*13*) confirmed Ball's findings regarding the occurrence of curly-top in sugar beets as a result of infestation by beet leafhoppers. He caged aphids, red spiders, thrips and beet leafhoppers on sugar beets and found that curly-top developed only in the beets on which the beet leafhoppers were caged. Shaw also reported that, even though no symptoms of the disease were visible when the beets were harvested the preceding fall, curly-top disease developed during the second season in beets planted for seed production, and that the symptoms of the disease were to be found primarily upon the young or growing leaves.

Bunzel (*7*) reported a high oxidase content in the tissues of diseased beets, recalling the high oxidase content reported in earlier experiments on tobacco mosaic.

Smith and Boncquet (*16*) reported that exposure of beets for five minutes to infestation by leafhoppers was sufficient to cause the symptoms of curly-top to develop, although two weeks or more might be necessary before the symptom would be obvious. They reported a bacillus as associated with the disease.

Boncquet and Hartung (*5*) reported beet leafhoppers not necessarily able to cause the disease while, if such leafhoppers were first fed on diseased beets and then allowed to feed on healthy beets curly-top would then develop.

Smith and Boncquet (*15*) reported the finding of bacteria in the diseased beets and confirmed the report of Boncquet and Hartung of the apparent inability of the leafhopper to transmit curly-top unless it had previously fed upon diseased plants.

Ball (*4*) urged the use of the name "curly-leaf" to clearly distinguish between the disease associated with leafhopper attack and any disease having symptoms in any way similar. He reviewed the status of curly-top or "curly-leaf," especially emphasizing the geographical relation of the breeding areas of the beet leafhopper to the areas in which severe outbreaks had occurred. He also emphasized the periodicity of migrations from breeding areas and suggested the probability that epidemics of curly-leaf could be predicted through the recognition of the periodicity of the flights of the insect. Commenting on the finding by Boncquet and Hartung that the insects were apparently unable to carry the disease unless they were fed upon diseased plants, he suggested the probability that some wild plant was acting as carrier of the disease.

Boncquet and Stahl (*6*) reported the occurrence of the curly-top infection in native vegetation.

Stahl and Carsner (*17*) reported that recently emerged beet leafhoppers, lifted from the plant before feeding were not carriers of curly-top, but became carriers of the disease when fed on diseased beets.

Severin (*10*) stated that the insects caught on wild vegetation produced the disease in beets in some cases while in other cases they did not. He listed wild plants from which the leafhoppers were reared and from which these insects transmitted curly-top to sugar beets.

Carsner (*8*) reported fourteen species of plants representing eight families as susceptible to curly-top.

Severin (*11*) demonstrated that after a nonviruliferous insect has fed on a diseased plant an incubation period for at least a few hours must elapse before this insect can transmit the virus.

Stahl and Carsner (*18*) reported that when an insect is once infected the infection is apparently permanent.

The appearance of sugar beets affected with curly-top in the different stages of the disease might well be more adequately described, but for the purpose of this paper brief mention of some of the conspicuous symptoms will suffice. When a plant is infected the mature leaves remain normal in appearance, the vein swellings and curling of the leaves appearing on only the new growth. There is no conspicuous color change in the early stages of the disease (Pl. 1). In the later stages the older leaves which were mature at the time of infection

turn yellow and die, while the younger central leaves which are dwarfed and usually show conspicuous vein swellings on the under sides remain green (Pl. 2, D). The vein swellings vary with different individual plants from cases where practically every veinlet is irregularly swollen (Pl. 2, B), to cases which show only scattered, inconspicuous, rounded swellings (Pl. 2, A). The disease is characterized by a necrosis of the phloem throughout the vascular system (Pl. 3, B), and in the advanced stages of the disease this necrosis shows in the roots as dark concentric rings (Pl. 4). The exudation of a clear, viscous liquid is often conspicuous on the oldest leaves which show the vein swellings (Pl. 2, C).[4]

DISTRIBUTION OF CURLY-TOP AND THE BEET LEAFHOPPER

The geographical distribution of the curly-top disease is limited to the arid or semiarid regions of the western part of North America in which the insect *Eutettix tenella* Baker is found and to the districts adjacent to these regions. In general it may be said, therefore, that the disease occurs wherever this leafhopper is found. It has been authoritatively reported from the following States: Arizona, California, Colorado, Idaho, Kansas, Nebraska, New Mexico, Oregon, Texas, Utah, and Washington.

The disease has been reported from practically every region in California in which beets are grown. The distribution of the beet leafhopper has been studied more extensively than has the distribution of the disease, and because of its significance from the standpoint of where the disease occurs or would occur if beets were planted the localities from which the insect has been reported are listed here.

LOCALITIES FROM WHICH THE BEET LEAFHOPPER HAS BEEN REPORTED

CALIFORNIA

1. Agatha.
2. Allensworth.
3. Alpaugh.
4. Altamont Pass.
5. Alvarado.
6. Angiola.
7. Antelope Valley.
8. Arlington.
9. Bakersfield.
10. Barstow.
11. Betteravia.
12. Bitterwater.
13. Blanco.
14. Brawley.
15. Buena Vista.
16. Buttonwillow.
17. Calexico.
18. Calipatria.
19. Castaic.
20. Castroville.
21. Centerville.
22. Chino.
23. Cholame.
24. Chowchilla.
25. Chualar.
26. Claremont.
27. Coalinga.
28. Compton.
29. Connor.
30. Corcoran.
31. Coyote Wells.
32. Crows Landing.
33. Davis.
34. Dixieland.
35. Dominguez Junction.
36. Dos Palos.
37. Earlimart.
38. El Centro.
39. Famoso.
40. Gonzales.
41. Goshen Junction.
42. Hamilton City.
43. Heber.
44. Helm.
45. Hemet.
46. Holtville.
47. Huntington Beach.
48. Huron.
49. Imperial.
50. King City.
51. Lancaster.
52. Le Grand.
53. Los Alamitos.
54. Los Baños.
55. Lost Hills.
56. McKittrick.
57. Manteca.
58. Marysville.
59. Mendota.
60. Merced.
61. Milpitas.
62. Modesto.
63. Mojave River.
64. Moro Cojo.
65. Mountain View.
66. Neenach.
67. Niland.
68. Norwalk.
69. Oro Loma.
70. Oxnard.
71. Pacheco Pass (entrance).
72. Paso Robles.
73. Pleasanton.
74. Prado.
75. Ravendale.
76. Red Bluff.
77. Riverside.
78. Rosamond.
79. San Ardo.
80. San Bernardino.
81. San Fernando Valley.
82. San Jacinto.
83. San Jose.
84. San Lorenzo Creek.
85. Santa Ana.
86. Santa Margarita.
87. Sargent.
88. Saugus.
89. Shafter.
90. Soledad.
91. Spence.
92. Spreckels.
93. Stockton.
94. Tejon Pass.
95. Tracy.
96. Victorville.
97. Visalia.
98. Volta.
99. Wasco.
100. Watts.
101. Westmoreland.
102. Williams.
103. Willowbrook.

[4] The progress of the work has been greatly aided by the use of the laboratory and field facilities of the Citrus Experiment Station of the University of California. The writers are indebted to the director and the staff for many courtesies and accommodations.

OREGON

1. Echo.
2. La Grande.
3. Pendleton.
4. Union.

WASHINGTON

1. Ellensburg.
2. Granger.
3. North Yakima.
4. Pasco.
5. Sunnyside.
6. Toppenish.
7. Wenatchee.

IDAHO

1. Blackfoot.
2. Buhl.
3. Burley.
4. Gooding.
5. Hansen.
6. Idaho Falls.
7. Jerome.
8. Nampa.
9. Oakley.
10. Payette.
11. Rupert.
12. Shelley.
13. Twin Falls.

NEVADA

1. Fallon.

UTAH

1. Austin.
2. Box Elder County.
3. Delta.
4. Elsinore.
5. Fillmore.
6. Garland.
7. Lehi.
8. Lynndyl.
9. Mills.
10. Monroe.
11. Moroni.
12. Nephi.
13. Ogden.
14. Panguitch.
15. Richfield.
16. St. George.
17. St. Joseph.
18. Salina.
19. Sigurd.
20. Spanish Fork.
21. Thompsons.

ARIZONA

1. Glendale.
2. Prescott.

COLORADO

1. Fort Collins.
2. Lamar.
3. Grand Junction.
4. Rocky Ford.

NEBRASKA

1. Grand Island.

TEXAS

1. Amarillo.
2. El Paso.

NEW MEXICO

1. Las Cruces.

The localities listed include those recorded by Ball (*1*), (*4*) and Severin (*11*) and unpublished reports by W. J. Hartung, G. T. Scott, and the present writers.

ECONOMIC IMPORTANCE OF CURLY-TOP

The studies on curly-top by the writers have been conducted in the main in California, and so the consideration of the economic importance here given is based principally on California conditions. The fact should be borne in mind, however, that the disease frequently causes serious losses in the beet-growing regions of other Western States. In the regions where beets have been grown in California the losses may in general be said to have varied (within limits) directly with the distance from the coast. In cooler coastal regions or so-called fog belts the damage is usually slight or relatively unimportant. As one goes inland into the progressively warmer and more arid districts the damage caused by the disease increases so that nearly every year the resulting losses are serious and in the so-called "bad years" the losses vary from very serious to total. The losses have been so serious in some of the interior districts that vast available areas otherwise suitable for beet growing have been completely abandoned after several years of unsuccessful efforts to grow beets on an extensive commercial scale. Curly-top was, of course, not the only difficulty encountered in these hot, dry districts, but, in the opinion of the writers, it was the limiting factor.

A more definite conception of the extent of the losses which the disease has caused may be formed from Table I. These figures were kindly furnished by sugar companies operating in California. They apply to districts which the writers have had under observation. The losses, therefore, are known to be due principally to curly-top. The yield per acre for these districts in years when curly-top has not been serious has ranged from 10 to 15 tons. The losses caused by the disease may be represented graphically by showing the net yield of beets, in tons per acre, in the four localities for each of the years considered (fig. 1).

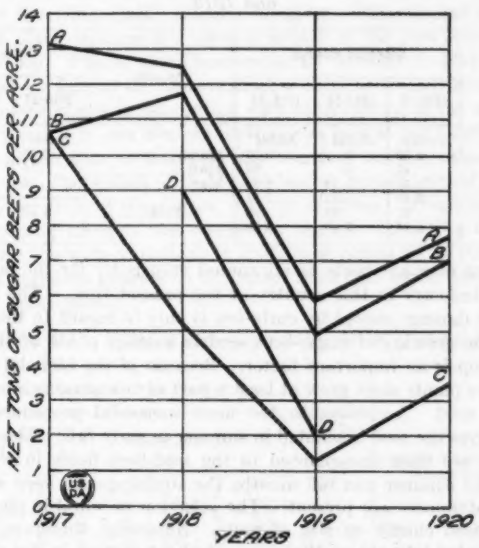

FIG. 1.—Graph showing the relative losses caused by curly-top disease

TABLE I.—*Losses caused by curly-top in four localities in California*

Locality and year	Crop area (acres)		Gross yield (tons)		Tare		Net yield per acre (tons)	Yield of sugar	
	Planted	Harvested	Harvested	Per acre	Per cent	Tons per acre		Per cent	Calculated tons per acre
District A:									
1917	1,307	1,287	17,774	13.8	5.0	0.7	13.1	19.3	2.5
1918	915	915	13,216	14.4	13.2	1.9	12.5	17.7	2.2
1919	1,288	1,288	8,349	6.5	8.2	.5	5.9	19.8	1.2
1920	933	933	7,882	8.4	8.2	.7	7.8	19.7	1.5
District B:									
1917	320	320	3,576	11.2	5	.6	10.6	19.4	2.1
1918	400	400	5,231	13.1	10.5	1.4	11.7	16.8	2
1919	675	675	3,748	5.6	8.13	.5	5.1	19	1
1920	490	470	3,601	7.7	8.4	.4	7.2	18.7	1.4
District C:									
1917	3,293	3,291	37,149	11.3	5.8	.6	10.6	19.2	2
1918	3,145	2,930	17,970	6.1	15.26	.9	5.2	16.8	.9
1919	3,425	2,550	3,664	1.4	9.18	.1	1.3	19.3	.3
1920	3,042	2,939	11,220	3.8	7.6	.3	3.5	19.8	.7
District D:									
1918	1,136	1,136	10,535.18	9.3	2.9	.3	9	17	1.5
1919	786	786	1,711.13	2.1	7.6	.2	2	17.1	.3

The question might be raised as to the possibility of other factors, such as variation in rainfall, having entered in to cause the great differences in yield. In answer to this question it may be said that irrigation is practiced in all the districts and that the amount of rainfall is therefore probably of no great significance. The rainfall is given, however, for a representative locality in the region of each of the districts A, B, and C to show the amount and distribution of the rainfall in the seasons of 1917, 1918, and 1919 (Table II).

TABLE II.—*Rainfall in the region of districts A, B, and C, seasons of 1917, 1918, and 1919*

Month	Seasonal rainfall			Month	Seasonal rainfall		
	1916–17	1917–18	1918–19		1916–17	1917–18	1918–19
	Inches	*Inches*	*Inches*		*Inches*	*Inches*	*Inches*
September	1.50	0	5.17	March	0.76	2.90	1.94
October	.37	0	.32	April	.15	.38	.08
November	.55	.71	2.39	May	.06	.09	0
December	2.40	.31	1.74				
January	.97	.47	.96	Total	8.92	7.31	15.66
February	2.16	2.46	3.66				

The growing of sugar beets as an annual crop is by far the more important phase of the industry in this country at the present time. The preceding discussion of the damage caused by curly-top is only in regard to this phase of the industry. The growing of sugar-beet seed is another phase of the industry in which curly-top is an important factor. Because of the biennial nature of the sugar beet, the plants must grow at least a part of two seasons in order to flower and produce seed. According to the most successful procedure under California conditions the seed is planted in summer or early fall. The young plants, or stecklings, are then transplanted to the seed-beet fields in winter or early spring. In the summer and fall months the stecklings are very apt to become infected if leafhoppers are present. The selection of suitable plants for transplanting is based chiefly on size of roots. Naturally, therefore, many plants which are infected late are on that account about normal in size and are transplanted to the seed fields. The result of this procedure is that a considerable proportion of the transplanted plants fail to produce flower stalks or produce only dwarfed, diseased stalks. An example of the part the disease plays was observed in the seasons of 1918 and 1919. The seed was planted late in the summer of the first year in a region infested with beet leafhoppers. As the stecklings grew up in the late summer and fall it was evident from the number of leafhoppers present and the cases of the disease scattered through the field that a considerable proportion of the plants had been inoculated. The stecklings were transplanted in the following winter and early spring with the result that because of curly-top about 30 per cent of the plants failed to produce seed.

No appreciable injury of seed beets due to infection in the second season of growth has been noted. When healthy stecklings are planted in the winter or early spring they grow to such an extent during the cool months before the leafhoppers appear or become abundant that there is practically no danger of injury from the disease. This observation is based on conditions in California, but it probably holds true also for other districts where seed beets are grown.

In addition to the direct loss due to the damage to the beet crops, great financial loss is involved when sugar factories are built in districts which prove unsuitable. The cost of building and later dismantling and removing the factory to another locality, together with the loss of capital invested in other improvements and equipment, must also be considered. Two instances in California alone are known in which factories were dismantled and removed because of the inability to grow beets on account of curly-top.

EFFECT OF CURLY-TOP ON GROWTH

A plant that becomes infected while young—when the root is perhaps 1 cm. or less in diameter—will usually die in a relatively short while or before the summer is past, but a plant that is about half grown or larger when infected will usually live a long time, as a rule to the end of the season.

Measurements made during two seasons of the roots of beets affected with curly-top show that increase in size of the root usually continues for a considerable length of time, often more than a month after the plant has become evidently diseased. The data taken during one season are listed in Table III. The plants were grown at Riverside, Calif. Figures are given of the size and number of the leaves of each plant, because the vigor of growth of the root is correlated with the size of the top. Plants which were evidently diseased on May 14 continued to grow, so that by July 26 the roots of most of them had nearly doubled in diameter. The measurements in connection with observations of the affected plants indicate that increase in size of roots continues as long as the older leaves, which were mature at the time of infection and therefore have remained apparently normal, survive. The weight of the top or leaves, as well as that of the root of each plant at the time of digging on July 26, is given. These figures indicate the great retarding effect of the disease on the growth of the tops. It is of interest to note the wide variations in individual plants as to the effect of the disease. The variation appears to be greater than that which occurs in the case of diseases of many plants.

TABLE III.—*Effect of curly-top on the growth of sugar beets*

Plant	Number of leaves on May 14	Maximum leaf and root dimensions							Weight on July 26		Date of observation and degree of disease when first noted
		Leaves on May 14		Root diameters							
		Width	Spread	On May 14	On June 2	Increase in 19 days	On July 26	Increase in 54 days	Top	Root	
		Cm.	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Gm.*	*Gm.*	
No. 3	10	10.2	43	2.09	3.57	1.48	3.98	0.41	27	78	May 14, two leaves affected.
No. 6	18	13.3	56	3.62	5.53	1.91	6.49	.96	62	207	May 14, five youngest leaves affected.
No. 8	16	11.4	52	3.38	5.51	2.13	5.71	.20	49.5	169	May 14, one leaf affected.
No. 12	15	15.9	53	3.10	4.98	1.88	5.85	.87	89.5	272	May 14, three youngest leaves affected.
No. 19	17	16.5	64	3.02	4.63	1.61	5.84	1.21	87	185	May 14, seven youngest leaves affected.
No. 20	15	12.7	62	3.80	5.02	1.22	5.20	.18	43.5	120	May 14, six of younger leaves affected.
No. 22	18	15	56	3.79	6.35	2.56	7.88	1.53	147	385	May 14, seven of younger leaves affected.
No. 32	15	13.3	62	2.98	4.50	1.52	5.26	.76	26	125	May 14, three of younger leaves affected.
No. 35	21	10.8	76	3.90	5.32	1.42	5.54	.22	74.5	114	May 14, nine of younger leaves affected.
No. 36	19	20.3	70	4.10	5.97	1.87	5.71	−.26	22.5	186	May 14, four of younger leaves affected.
No. 38	12	10.8	63	2.41	3.08	.67	2.88	−.20	10	20.5	May 14, five of younger leaves affected.
No. 2	14	10.2	46	2.35	4.70	2.35	7.12	2.42	132	276	June 2, one young leaf affected.
No. 4	8	6.4	47	1.19	3.02	1.83	4.54	1.52	55	112	June 2, four of younger leaves affected.
No. 5	15	17.8	51	2.77	5.56	2.79	7.63	2.07	153	417	June 2, eight youngest leaves affected.
No. 10	10	8.3	58	1.23	3.10	1.87	3.60	.50	22	48.5	June 2, six youngest leaves affected.
No. 11	19	15.9	61	4.62	7.49	2.87	8.06	.57	90	440	June 2, four youngest leaves affected.
No. 13	11	14	53	3.25	5.78	2.53	6.94	1.16	175	380	Do.
No. 14	10	15	66	3.03	5.60	2.57	7.67	2.07	300	440	June 2, five of younger leaves affected.

TABLE III.—*Effect of curly-top on the growth of sugar beets*—Continued

Plant	Number of leaves on May 14	Maximum leaf and root dimensions: Leaves on May 14 Width	Leaves on May 14 Spread	Root diameters On May 14	On June 2	Increase in 19 days	On July 26	Increase in 54 days	Weight on July 26 (grams) Top	Root	Date of observation and degree of disease when first noted
		Cm.	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Gm.*	*Gm.*	
No. 16..	24	12.7	66	3.99	7.28	3.29	7.61	0.33	52	252	June 2.
No. 23..	12	14.6	60	2.72	5.25	2.53	6.70	1.45	83	207	June 2, two youngest leaves affected.
No. 26..	20	17.8	61	4.69	8.69	4.00	10.82	2.13	185	753.5	June 2.
No. 27..	19	14	69	3.89	6.76	2.87	8.72	1.96	152.5	449	June 2, three of younger leaves affected.
No. 28..	16	20	74	3.61	6.40	2.79	8.20	1.80	169	300	June 2, seven of younger leaves affected.
No. 31..	15	16.5	81	4.15	6.99	2.84	8.13	1.14	19	425	June 2, about half of the leaves affected.
No. 37..	9	8.3	55	1.60	3.36	1.76	4.32	.96	22	64	June 2, one young leaf affected.
No. 9....	16	16.5	69	3.93	7.90	3.97	10.23	2.33	321	802	June 2, healthy.
No. 15..	12	14.6	77	2.69	5.25	2.57	6.71	1.46	68	300	Do.
No. 17..	13	21.6	79	5.83	9.76	3.93	10.95	1.19	301.5	896.5	Do.
No. 24..	13	10.2	53	2.18	4.79	2.61	8.24	3.45	225	438	Do.
No. 30..	13	10.2	48	3.11	5.22	2.11	6.46	1.24	116	306.5	Do.

DRY WEIGHT OF TOPS INCREASED

Moisture determinations which were made at three different times of the tops or leaves of young healthy and diseased beets show that the proportion of dry weight of the tops of young diseased plants exceeds the proportion of dry weight of those of young healthy plants by 2.5 to 3 per cent. This excess of dry weight is attributed to the excessive accumulation of carbohydrates, probably sugars. Due to the breaking down of the phloem, normal translocation is impossible. The clear, viscous exudate (Pl. 2, C) has been repeatedly found by Fehling's test after hydrolysis with an acid to contain a complex sugar, probably saccharose. In only one or two instances were traces of reducing sugars detected. The escape of the exudate by rupturing the tissue is attributed to the high pressure developed by the abnormal accumulation of the sugars.

TABLE IV.—*Artificial inoculations of sugar beets, resulting in no disease* [a]

Date	Kind of inoculum	Kind and number of plants inoculated	Method of inoculation
Season of 1918:			
March 3....	Fresh excreta of the leaf hopper.	Two young beet seedlings.	Point of steel needle dipped into drops of excreta and then pricked into plants.
August 3...	Ringer's solution extract and aqueous extract of young leaf of curly-top beet.	Four young beet seedlings.	Sprayed each extract on two plants; then caged on each five nonviruliferous leafhoppers.
August 12..	Six adult leafhoppers macerated in 2 cc. of Ringer's solution.	Three young beets.	A drop of inoculum placed on each of two leaves of each plant and pricked in with small steel needles.
Do.....	Curly-top beet leaf macerated in Ringer's solution.	.....do.....	Do.
Do.....	Curly-top beet leaf macerated in distilled water.	.....do.....	Do.
Season of 1919:			
May 24.....	Pieces of curly-top beet leaf...	Eight beets (these had five or six true leaves).	Placed tissue on midrib near base of blade, macerating over and pressing into a slit in the midrib.
August 8...	Pieces of petiole of leaf of curly-top beet.	Seven young beets.	Placed tissue on center of blade of one leaf of each and then macerated into wound in the blade with scalpel and needle.

[a] Since this manuscript was prepared curly-top has been artificially communicated by transferring juice from diseased to healthy beets, as described in the following publications: SEVERIN, H. P. CURLY LEAF TRANSMISSION EXPERIMENTS. Phytopathology 14: 80–93, illus. 1924: CARSNER, E., and STAHL, C. F. PROGRESS REPORT ON CURLY-TOP OF THE SUGAR BEET. (Abstract) Phytopathology 14: 122–123. 1924.

TABLE IV.—*Artificial inoculations of sugar beets, resulting in no disease*—Contd.

Date	Kind of inoculum	Kind and number of plants inoculated	Method of inoculation
Season of 1919—Continued.			
August 8	Entire viruliferous leafhopper.	Seven young beets.	One insect macerated on and pricked into a leaf of each plant. Insect first stunned by a gentle pressure between the fingers.
August 13	Two entire curly-top plants macerated in distilled water.	Three young beets.	About 500 cc. of the extract poured on the soil around the roots.
Do	Part of the preceding extract.	Several young plants.	Plants dug with as little injury to the roots as possible and roots placed in extract for one hour; older leaves then removed and plants replanted.
August 27	Distilled-water extract of two entire curly-top beets.	Several beet plants with roots one-fourth inch in diameter.	Roots placed in the extract for one hour; older leaves then removed and plants replanted.
October 2	Pieces of midrib of badly affected leaf of a curly-top beet.	One young beet	Tissue placed in a slit in the petiole; wound not covered.
Do	Piece of midrib of curly-top beet leaf.	One beet seedling	Tissue placed in a slit in the hypocotyl; wound covered with soil.
Do	A leafhopper nymph from a curly-top beet.	One young beet	Insect forced into a slit in the petiole of the plant; wound bound with cloth and grafting wax.
Do	Adult leafhopper from a curly-top plant.	One beet seedling	Insect pressed into a slit in the hypocotyl; wound wrapped and covered with grafting wax.
Do	Entire viruliferous insect	Three beet plants with ¼-inch roots.	One insect in a slit in the crown of each plant just below the ground level.
October 18	Small piece of petiole of a leaf of a curly-top beet.	Three young beets.	Tissues placed in a slit in the root just below ground level; wound covered with soil.
October 21	Tops and roots of curly-top beets separately extracted in distilled water.	Eight plants with ¼-inch roots.	Roots allowed to stand in extract over night; leaves then removed and roots replanted.
Season of 1920:			
April 27	Extract of entire curly-top plant in distilled water.	Several beets	Healthy leaves cut and cut ends placed in vials of the extract; nonviruliferous leafhoppers placed on these for two days and then on healthy beets.
May 2	Tap-water extract of two entire young curly-top beets which had been macerated in a mortar.	Three young beet plants	Slowly injected through fine glass nozzles attached to a funnel containing the extract, which was so elevated as to cause a gentle pressure of the liquid.
May 12	Tap-water extract of two entire curly-top beets which had been macerated in a mortar.	Two young beet plants.	Cut ends of petioles of healthy leaves placed in vials of the extract; seven nonviruliferous insects were then caged on the blades of these leaves for three days and then transferred to healthy plants.
May 15	Expressed juice of 12 young curly-top beets.	Several young beets.	Plants dug and roots allowed to stand in the juice for four hours and then replanted.
Do	Expressed juice of curly-top plants and expressed juice diluted with equal parts of tap water.	——do——	Roots placed in the two solutions for four hours and then replanted.
Do	——do——	——do——	Cut ends of petioles of healthy leaves placed in vials of the extract and nonviruliferous insects placed on them for four days and then transferred to healthy beets.
July 5	Parts of viruliferous leafhoppers which had been anæsthetized with ether to facilitate dissection.	Twenty-one young beets.	Parts of insects (e. g., heads, thorax, and abdomen) were placed in Ringer's solution, then on each plant an insect part was inoculated into the base of the blade of each of the first true leaves by means of a sharp steel needle.
Season of 1921:			
May 3	Juice of a curly-top plant extracted by means of a hypodermic syringe.	Seven beet plants. One had only cotyledons; the others had seven leaves.	Juice slowly injected by means of hypodermic syringe immediately after it was extracted from diseased plant. Injected into crowns and cut end of a petiole.

TABLE V.—*Inoculation of sugar beets with insects other than Eutettix tenella, no disease resulting*

Season, number, and date	Species of insect	Number of insects used	Length of time insects were on curly-top beets	Healthy plant to which the insects were transferred	Length of time insects were left on healthy plants
Season of 1915:					
No. 1, Aug. 30	Empoasca viridescens	5	4 days	Young beet	28 days.
No. 2, Aug. 30	Agallia spp	2	do	do	Do.
No. 3, Aug. 30	Thamnotettix geminatus (?).	1	do	do	Do.
No. 4, Aug. 30	Cicadula 6-notata	2	do	do	Do.
No. 5, Aug. 30	Liburna sp	1	do	do	Do.
No. 6, (?)	Empoasca viridescens	(?)	Collected from diseased plants.	do	22 days.
No. 7, (?)	Agallia spp	(?)	Collected from diseased plants in the field.	do	Do.
Season of 1917:					
No. 8, Aug. 29	Empoasca viridescens	7	47 days	(a)	47 days.
No. 9, Aug. 29	Agallia cinerea	7	35 days	(a)	35 days.
No. 10, Sept. 4	Empoasca viridescens	6	39 days	(a)	39 days.
No. 11, Sept. 4	Nysius sp	6	29 days	(a)	29 days.
No. 12, Sept. 4	White fly (Aleyrodidae)	12	do	(a)	Do.
Season of 1917:					
No. 13, Sept. 14	Aphis sp	Large.		(2) (b)	
No. 14, Sept. 14	do	Large.		(3) (c)	
No. 15, Sept. 4	Lygus pratensis	10	29 days	(a)	29 days.
Season of 1919:					
No. 16, May 22	Acinopterus sp	3	4 days	Young beet	15 days.
No. 17, May 22	Chlorotettix sp	3	do	do	Do.
No. 18, May 22	Agallia sp. (large)	(?)	do	do	Do.
No. 19, May 22	Agallia cinerea	3	13 days	do	44 days.
No. 20, May 22	Empoasca viridescens	5	20 days	do	37 days.
No. 21, July 1	Thamnotettix montanus	4	4 days	do	27 days.
No. 22, (?)	Aphis sp	Large.	3 days	(d)	
No. 23, Apr. 28	Eutettix strobi	3	2 days	Young beets	2 days.
Season of 1920:					
No. 24, Feb. 23	Empoasca viridescens	6	Several days	Young beets and *Stellaria media*.	18 days.

[a] The insects were placed in a cage with a small curly-top beet and a small healthy beet for the number of days stated.

[b] A curly-top beet leaf heavily infested with aphids was placed in a cage with healthy and curly-top plants. The insects colonized on both plants.

[c] A curly-top beet leaf heavily infested with aphids was placed in a cage with a healthy plant upon which the insects colonized.

[d] A curly-top leaf with aphids on it was removed to a cage with a healthy beet and another to a cage with two healthy *Stellaria media* plants.

Observational evidence agrees with these negative results from attempts to transmit the disease by insects in so far as aphids and *Empoasca viridescens* are concerned. These two species have been repeatedly observed infesting the plants in cages which contained both curly-top and healthy plants and in no case has there been any evidence that they transmitted the virus of the disease.

The question of transmission by grafting perhaps should be considered in this connection. Smith and Boncquet (*15*) claim to have transmitted the disease by grafting parts of diseased beets on to healthy plants. Thus far the present writers have been unable to successfully graft parts of a curly-top plant on to a healthy plant, and the attempts to make these grafts have not produced the disease.

IS THERE AN INCUBATION PERIOD IN THE LEAFHOPPER?

As has previously been stated, *Eutettix tenella* is the only known agent or means by which the virus of curly-top can be transmitted. Also, the fact has been pointed out that the ability to produce the disease is not an inherent characteristic

of the insect. In view of these facts the question as to whether or not an appreciable interval of time must elapse after a nonviruliferous [5] leafhopper has fed on a diseased plant before it is able to effectively transmit the virus may have some significance regarding the nature of the virus. Smith and Boncquet (*15*) state that a period of at least 24 hours but not more than 48 hours must elapse. Severin (*11*) worked with batches of 25 to 50 insects instead of single leafhoppers. He found that at high temperatures (about 100° F.) the insects were occasionally able to produce the disease within four to six hours after they had been placed on a diseased plant. For this short period his results showed 8 cases of disease from 60 tests. No positive results were obtained from 36 tests for a period of one to three hours. Periods of 24 to 48 hours after the insects were placed on the diseased plant gave a greater proportion of positve results than shorter ones. The experimental tests which the writers have thus far made in regard to this question are listed in Table VI. In each of these experiments nonviruliferous leafhoppers were placed on a curly-top beet for a given number of hours and then transferred individually to young healthy plants for a certain number of hours and then transferred again, etc. For example, in experiment No. 1 the nonviruliferous insects were caged on a curly-top plant for 24 hours and then caged singly on healthy plants for the same length of time. The first period tested was from 24 to 48 hours after the insects were placed on the diseased plant. After that the insects or those which still survived were transferred to other healthy plants for 24 hours. This second period, therefore, was from 48 to 72 hours after the insects were placed on the diseased plant. The successive transfers of the same insects are indicated in Table VI by attaching the letters *a*, *b*, *c*, etc., to the number of the experiment.

The results show that some of the insects were able to transmit the virus within a period of 21 hours and 45 minutes after they were placed on the diseased beet. In another case the period was 22 hours and 20 minutes, and in a third case it was 23 hours. In some cases the insects all failed to produce the disease within periods of 24 hours or less. A point which may be of some significance is that the results seem to indicate that a greater number of the insects become able to transmit the virus after a longer period than 24 hours than are able to do so in the shorter time. The facts cited seem to indicate that a multiplication of the causal agent takes place within the insect.

RELATION OF THE NUMBER OF INSECTS TO CURLY-TOP

Comparative inoculations were made at the same time and on similar plants with different numbers of insects. The object was to determine whether or not the quantity of virus injected was significant in producing the disease or in regard to the period of incubation. Twelve experiments have been performed to test this. The procedure in these tests was so varied that the details can not be readily tabulated. The results, however, may be summarized. They indicate that when 10 insects are used the inoculated plant is more likely to become diseased than when the inoculation is made with 1 or 2 insects. It seems probable that this fact is due to the variation of individual insects in regard to infection rather than to the quantity of virus injected. In the cases where the disease is produced there is no difference in the incubation period or severity of the effect between inoculations with single insects and those with 10 insects.

[5] It has recently been brought to the writers' attention that the term *viruliferous* occurs in medical literature; and as it appears to have the same meaning *viruliferous* (meaning virus-bearing) as used by the writers in previous publications, it is of course desirable to use *viruliferous* for the sake of preventing a duplication of terms.

TABLE VI.—*Length of incubation period in the leafhopper before becoming viruliferous*

Date started	Experiment No.	Length of time			Number of insects		Remarks
		On curly-top plant	On healthy plants	Total	Tested	Producing the disease	
Season of, 1918:		*Hours*	*Hours*	*Hours*			
July 17	1a	24	24	48	20	1	Inoculated plants in 5-inch pots.
Do	1b		24	72	14	5	Plants in bed in green-house. More favorable conditions for growth may have been a factor.
Do	2a	24	24	48	20	5	
Do	2b		24	72	20	9	One insect which produced the disease in the first period failed to do so in the second period, so that five more than in the first period were then effectively viruliferous.
August 14	3a	24	24	48	30	5	
Do	3b		24	72	30	6	
September 2	4a		24	48	30	10	*Stellaria media* plants were used instead of beets.
Do	4b		24	72	28	22	One insect which caused disease in the first period did not cause it in the second, so there was in the second period an increase of 13 that were effectively viruliferous.
Do	4c		24	96	26	15	
November 8	5a	5	18	23	26	11	*Stellaria media* plants were used instead of beets.
Do	5b		73	96	24	22	The second period was so much longer than the first that a fair basis of comparison is hardly afforded.
Season of 1920:							
August 23	6a	3	19½	22½	5		
Do	6b		9	31½	5		
Do	6c		15	46½	5	1	
Do	6d		72	120	5	1	
Do	7a	7	15½	22½	4	1	
Do	7b		9½	31½	4	3	
Do	7c		15	46½	4		
Do	7d		72	120	4	2	One of the four insects did not produce the disease at all.
September 13	8a	5½	17	22½	10		
Do	8b		7½	30	10	1	Insect No. 8 produced the disease.
Do	8c		17½	47½	10	3	Nos. 5, 7, and 10, produced the disease.
Do	8d		144	192	10	4	Nos. 6, 7, 9, and 10, produced the disease; four of the insects did not produce the disease at all.
September 14	9a	4½	17½	21½	10	2	Nos. 2 and 7 produced the disease.
Do	9b		7½	29½	10		
Do	9c		72	96	10	3	Nos. 2, 5, and 7 produced the disease; seven of the insects did not produce the disease at all
September 22	10a	6	2½	240	10		
Do	10b		15	23½	5		
Do	10c		8	31½	10	1	No. 10 produced the disease.
Do	10d		6	47½	10	2	Nos. 1 and 6 produced the disease.
Do	10e		8	55½	10	1	No. 2 produced the disease.
Do	10f		16½	72	10		
Do	10g		240	312	10	4	No. 1, 4, 6, and 10, produced the disease; five of the insects did not produce the disease at all.

VARIATION OF INDIVIDUAL INSECTS IN REGARD TO INFECTION

In the attempt to determine whether or not there is any uniformity of behavior among individual leafhoppers in regard to producing the disease, a considerable number of insects were caged singly on individual plants, under similar conditions, on beets or other susceptible plants for periods of 24 hours or longer on consecutive days. Tests of this nature were repeatedly made, using 3 to 30 individual insects in a test. The results of two of these tests or series are brought together in Table VII.

The question has been raised as to whether the infectivity of the insect or ability to produce the disease runs in cycles, such as might occur if the virus were an organism with a definite life cycle. The results as listed in Table VII seem to give no evidence of definite cycles of infectivity. The evidence seems to justify the conclusion that, in general, when an insect has produced or failed to produce the disease in a given period it is more likely to give a similar result in the subsequent one or more periods before changing to the other result. In other words, the positive or negative results are more likely to be grouped together in consecutive periods than they are to alternate. This idea is based on the results shown in the table, as well as on the results of other tests. As to what is the significance of this seeming tendency of the results, the evidence thus far justifies only speculation. It is of interest to note the preponderance in the number of cases in which the disease was produced over the number of negative results.

TABLE VII.—*Variation of individual insects in regard to causing infection*

[The plus sign (+) indicates the production of the disease, and the minus sign (−) indicates that no disease resulted]

Inoculation period	Insect No.																														Total for each period	
Hours	6	7	9	12	13	21	22	53	54	55	56	57	58	59	60	61	63	65	66	67	68	69	71	72	73	86	87	88	89		+	−
24	+	+	+	+	+	+	−																								6	1
384	+	+	+	+	−	+	+																								6	1
24	−	+	+	+	+	+	+																								6	1
24	+	+	+	+	+	+	+																								7	0
24	+	+	−	+	+	+																									5	2
24	+	+	+	+	+	+																									6	0
24	+	+	+	+	−	−																									4	2
24		−	−	−	−																										0	4
48	+	−	−	−																											1	4
24	−	−	+	+		+																									3	2
24	−	+	−	+	−																										2	3
24	+																														1	0
24								−	−	−	+	−	+	+	+	+	−	−	−	+	−	+	+								8	8
48								−	−	+	−	+	+	+	+	+	+	−	+	−	+	+	+								10	6
24								+	−	+	−	+	−	+	−	+	−	+	−	+	−	−	+	−	+	−					8	9
24								+	−	+	−	−	−	−	−	−	+	−	+	−	+	−	−	+	−						5	13
24								+	+	−	+	+	+	+	+	+	+	+	+	+	−	+	−	−	+						14	3
72								+	+	−	+	−	−	+	+	+	+	−	+	−	+	+	−		+						11	2
72								+	−	−	+	+	−	+	−	+	−	+	−	+	−	+	−		+						8	5
72									+	−	+	−	−	−	−	−	+	−	+	−	+	−	−		+						4	7
24									−	−	+	−	−	+	−	+	−	−	−	+	−	−	+		+						5	6
48											−	+	−	+	−	+	−	+	+	−	+	+	−		−						7	3
48											+	+	−	+	−	+	−	+	−	+	+	+	−		−	−	+	−	+	−	10	3
48											+	+	−	+	−	+	−	+	−	+	+	+	−		+	+	+	+	+	+	13	1
48											−	−	−	−	−	−	+	−	+	−	+	+	−		+	+	−	−	+	−	6	8
120											+	−	−	+	−	+	−	+	−	+	−	−	−		+	−	−	−	+	+	7	7
24											−	+	−	+	−	+	−	+	−	−	−	+	−		+	−	+	−	+	−	7	5
24											+	−	−	+	−	+	−	+	−	+	−	+	−		+	+	−	−	+	−	6	5
48												−	−	+	−	+	−	+	−	+	−	+	−		+	−	+	−	+	−	7	4
72													−	−	−	−	+	−	+	−	+	−	−		+	−	−	+	+	+	6	5
24													+	−	−	−	+	−	+	−	−	−	−		+	−	+	−	−	−	4	7
72													−	−	−	−	−	+	−	+	+	−	+		−	−	−	+	−	−	3	7
168													+	−	+	−	−	+	−	−	−	+	−		+	−	−	−	−	−	4	5
24													+	−	+	−	+	+	−	−	−	−	−		+	+	+	−	+	−	8	2
216													−	−	−	−	+	+	−	+	−	−	−		+	+	−	+	−	−	7	4
24													+	−	+	−	−	−	−	−	+	−	−		+	+	−	−	−	−	5	2
Total for each insect:																																
Plus	8	8	7	8	5	7	4	5	3	4	2	11	12	4	15	5	12	9	1	17	5	18	2	19	8	7	1	10	3	220		
Minus	3	3	4	3	2	4	1	2	6	2	4	5	10	1	7	1	10	12	5	6	17	5	3	2	13	6	5	2	3			147

The numbers at the top of the columns were used to designate the individual insects. The figures in the left-hand column show the number of hours the insects were on the plants. The plus sign (+) indicates that the disease was produced as a result of the inoculation in that period. Likewise the minus sign (−) indicates that

curly-top was not produced in a given period. The periods represented between any two of the horizontal lines show the number of insects used on any one day or period. Where the columns are very short the fact is to be inferred that the insect concerned soon escaped or died. A blank in a column indicates that no note was obtained for that period, owing to the fact that the tag label could not be found.

RELATION OF LIGHT TO INFECTION

A limited amount of experimental work has been done in the attempt to determine whether or not light is necessary for infection to occur. Young beet plants were kept in darkness for a period and then inoculated in comparison with a series of plants exposed to daylight. In each experiment some of the plants were inoculated on the cotyledons and others on the true leaves. The details of the procedure and the results are listed in Table VIII.

TABLE VIII.—*Relation of light to infection of beet plants tested in 1920*

Experiment and date	Length of time			Point of inoculation	Number of plants inoculated	Diseased
	In the dark before inoculation	Insects on plants	In the dark			
	Hours	*Hours*	*Hours*			
No. 1, Mar. 24	48	72	120	Cotyledons	5	[a] 2
	48	72	120	First true leaves	5	[b] 1
No. 2, Apr. 1	26	22	48	Cotyledons	3	3
	26	22	48	First true leaves	3	1
No. 3, Apr. 24	34	21	55	Cotyledons	8	3
	34	21	55	First true leaves	7	1
	[c]	21	----------	Cotyledons	8	2
	[c]	21	----------	First true leaves	7	4
No. 4, Nov. 25	19	26	65	Cotyledons	6	6
	19	26	65	First true leaves	6	3
	[c]	20	----------	Cotyledons	4	4
	[c]	20	----------	First true leaves	4	4
No. 5, Dec. 4	22	20	42	Cotyledons	4	2
	22	20	42	First true leaves	4	0
	[c]	20	----------	Cotyledons	4	1
	[c]	20	----------	First true leaves	4	2

[a] Only four lived. [b] Only two lived. [c] Not placed in the dark.

In each test plants of the same age were used throughout, and they were grown under conditions as nearly similar as possible except in regard to light. If any significance may be attached to the results of these few tests, they indicate that, with young plants which have been kept in darkness for about 24 hours prior to inoculation, infection can be more readily effected on the cotyledons than on the leaves. Plants exposed to usual daylight, on the other hand, seem about as easily infected on the true leaves as on the cotyledons. If these facts be true, it would seem logical to presume that the presence of food substance as stored products in the cotyledons or from the process of photosynthesis in all green parts facilitates infection. It seems worthy of note that in test No. 1, where the plants were kept in darkness for five days, only two of the five leafhoppers caged on the true leaves survived, while all five of those on the cotyledons lived. Probably those on the true leaves were starved.

EFFECT OF HEAT ON THE VIRUS

The question as to whether or not the virus of curly-top is destroyed by relatively high temperatures was studied by placing nonviruliferous leafhoppers on parts of curly-top beets which had been immersed in water at the desired temperature. The root pieces were cut so that in no case was the thickness greater than 1 cm. A period of 10 minutes was used in each experiment. After the leaves or root pieces had been immersed in the heated water, nonviruliferous insects were caged on these beet parts for 24 hours and then removed to healthy plants. The results of the experiments are shown in Table IX. It should be noted that in the cases where the insects failed to secure the virus from heated leaves the leaves were so decomposed by heat as to be unfit for food. The results of the experiments seem to indicate that the virus is not destroyed by temperatures lower than those at which the beet tissue is killed. Many bacterial parasites of plants are killed by temperatures lower than those which the curly-top virus will endure.

TABLE IX.—*Effect of the application of heat on the virus of curly-top*

Season, experiment, and date	Temperature of water used (°C.)	Parts of diseased plant heated	Results
Season of 1919:			
Number 1, April 29	45 to 46	Leaves	Plants diseased.
Number 1, April 29	54 to 55	do	Plants healthy.
Number 2, May 8	45	Leaf	Plants diseased.
Number 2, May 8	50	do	Plants healthy.
Number 3, June 6	49 to 50	Leaves	Plants diseased.
Number 4, August 7	49.5 to 50	do	Do.
Number 4, August 7	51 to 52	do	Do.
Season of 1920:			
Number 5, April 14	51 to 52	do	Do.
Season of 1921:			
Number 6, October 14	51 to 52	Leaves and root pieces	Do.
Number 6, October 20	52 to 53	do	Do.
Number 6, October 20	60 to 61	Leaves	Plants healthy.
Number 6, October 20	60 to 61	Root pieces	Plants diseased.

EFFECT OF DESICCATION ON THE VIRUS

Four tests in which dried tissue of curly-top beets was remoistened with water and then placed in cages as food for nonviruliferous leafhoppers have given only negative results. The insects were unable to produce curly-top when removed to healthy plants. The survival of the insects in three of the tests for periods of approximately 24 hours at room temperature indicates that they must have fed to some extent on the old diseased tissue. Further work is needed to justify the conclusion that the virus is destroyed by desiccation.

DISTRIBUTION OF VIRUS IN AFFECTED PLANTS

Those leaves which are mature at the time of infection remain normal or free from symptoms of the disease until they begin to yellow with age. The question has therefore been suggested as to whether or not these apparently normal leaves contained the virus or disease-causing factor. Tests have been made by the use of nonviruliferous leafhoppers. These insects were caged on the leaves to be tested and then transferred to healthy beet plants to determine if they had become effectively viruliferous. Eight trials of this nature have been made. In a few instances the insects failed to secure the virus from the old, normal-appearing

leaves. In most of the trials, however, the insects secured the virus from the normal-appearing leaves as well as from the younger, evidently diseased leaves. The peculiar fact should be noted, however, that the insects which were placed on the leaves showing the symptoms were able to transmit the virus sooner than those insects which were placed on the older, normal-appearing leaves.

The fact that the virus is present in the roots of diseased plants has been repeatedly determined by the use of nonviruliferous insects.

IS A BACTERIAL ORGANISM ASSOCIATED WITH CURLY-TOP?

The discovery of a bacterial organism associated with beets affected by curly-top was announced by Smith and Boncquet (*15*) and earlier workers. The work of the present writers on this point may be divided into two phases. In the first place, attempts have been made to grow beet plants under aseptic conditions by the use of steamed soil and disinfected seed. The plants were covered with bell jars to protect them from contamination by air-borne organisms. After curly-top had been produced in some of the plants, pieces of affected leaves of these were transferred, without disinfection, to tubes or plates of a culture medium. Bacteria grew from the leaf pieces in some instances, but the important phase of the results is that in several of the cases the medium into which the curly-top tissue was introduced remained sterile or showed only fungus contamination.

The other phase of the study of the question has been the plating of pieces of tissue of curly-top plants. Pieces of the interior of roots and of petioles have been cut out with aseptic precautions and then plated without disinfection. Pieces of diseased leaves have been plated after disinfection with mercuric chlorid for 5 to 15 minutes. In the majority of the cases both the untreated and the disinfected tissue remained sterile. In some instances bacteria grew from the tissue pieces, but several different types were represented. None of these were tested by inoculation.

The results of these two lines of work indicate at least that no easily culturable specific bacterial organism is constantly associated with curly-top. That bacteria may get into curly-top tissues through the open wounds caused by the dying back of lateral roots and the ruptures on the leaves made by the liquid exudate seems probable.

THE INCUBATION PERIOD OF THE DISEASE

The length of time that elapses from the inoculation of the plant until the first symptoms appear is considered as the incubation period of the disease. Temperature affects the length of the period, as is noted elsewhere in this paper. The incubation period is shortest when, with high temperatures, conditions are still favorable for growth of the plant. With beet plants in cheesecloth cages out-of-doors in the summer at Riverside, Calif., as short an incubation period as four days has been occasionally noted. With *Stellaria media* an incubation period of four days has several times been observed. In one case with this plant symptoms were visible within 48 hours after inoculation. In general the incubation period in sugar beets varies from 7 to 14 days. With low temperatures or conditions otherwise unfavorable for growth the period may be prolonged, sometimes for a month or more.

The discussion of the incubation period of the disease as given here should not be confused with the discussion by Severin (*11*). He speaks of the time elapsing between inoculations and when "noninfective" insects were able to secure the causative agent from the plant as the incubation period of the causative agent.

RELATION OF TEMPERATURE TO CURLY-TOP

The writers' ideas as to the relation of temperature to the incubation and development of curly-top are based principally on observations made under natural conditions at different seasons. Facilities for accurately controlling temperatures where plants were grown in California have not been available, but the evidence obtained indicates that soil temperature as well as that of the air affects the duration of the incubation period in beets. For instance, in an experiment where all the inoculated plants were exposed to the same range of air temperatures but were exposed to two different soil temperatures, symptoms of the disease appeared first at the higher temperature. With the plants where the maximum soil temperature ranged from 25° to 35° C. symptoms of curly-top appeared on the eighth day, while in the case of the plants where the maximum temperature varied from 15° to 20.5° C. symptoms first appeared on the eleventh day. Results of two series of tests conducted with controlled soil temperatures in a greenhouse at Madison, Wis.,[6] support the view as to the influence of soil temperatures. Plants inoculated in California and sent by mail to Madison and there grown at a soil temperature of 33° C. developed the symptoms sooner than did those at 15° C. In the case of *Stellaria media* the results of one test indicate that soil temperature is less important than in the case of the beet. Where the air temperature was the same for both sets of plants the symptoms appeared at the same time in both the high and low temperature soil. If this seeming difference between beets and chickweed holds true it may possibly be explained on the basis of the different structure of the two plants. In the case of the beet the virus has to pass through the crown of the plant, which is usually at or below the soil level, in order to reach the new leaves. The main shoots of a *Stellaria media* plant throw out lateral branches, so that the curly-top virus may reach new leaves without necessarily passing through the root or crown. It would, therefore, probably be influenced in its rate of spread or development chiefly by the air temperature.

Low temperatures retard the progress of the disease as they lengthen the incubation period. This fact probably explains why curly-top is generally less injurious, even when it occurs in the cooler coastal districts, than it is in the warmer regions.

AGE OF PLANTS IN RELATION TO SUSCEPTIBILITY

The question of the relation of the age of a plant to its susceptibility to curly-top infection is of great interest from the standpoint of cultural practice as to time of planting. Field observations indicate that if beets have attained a considerable size before the leafhoppers appear they are much more likely to make a harvestable crop than they will if very small when the leafhoppers come in. This fact seems due, in part at least, to the beets becoming less easily infected as they grow older. A limited amount of experimental work on the question, which is detailed in Table X, supports this idea. In the experiments where plants of different ages were inoculated on the same date both lots were growing under similar conditions. The age of the plants used is indicated in the table by giving the number of leaves developed.

[6] The details of the tests at Madison were handled by Hurley Fellows.

TABLE X.—*Age of sugar beet plants in relation to susceptibility*

Season, experiment, and date	Age of plants	Insects per plant	Number of plants	
			Inoculated	Diseased
Season of 1920:				
No. 1, January 2	6 to 8 leaf stage	1	35	22
No. 2, March 13	Cotyledon stage	1	6	2
	6 to 7 leaf stage	1	6	0
No. 3, March 17	Cotyledons and first pair of leaves	1	6	4
	7 to 8 leaf stage	1	6	1
No. 4, March 31	Cotyledons and first pair of leaves	1	15	8
	6 to 8 leaf stage	1	15	1
No. 5, April 2	Cotyledons and first pair of leaves	1	15	12
	6 to 8 leaf stage	1	15	3
No. 6, July 14	5-months old	2	20	0
Season of 1921:				
No. 7, July 2	4 months old	2	10	1
	2 months old	2	11	2
No. 8, September 20	8 to 9 leaf stage	2	6	1
No. 9, October 14	10 to 16 leaf stage	2	14	1

OTHER PLANTS SUSCEPTIBLE TO CURLY-TOP

A list of plants found susceptible to curly-top has been previously given (*8*). Since that time no extensive effort has been made to determine what other plants may be affected by the disease. The annual (redmaids) *Calandrinia menziesii* T. and G., may now, however, be added to the list of those plants proved to be susceptible (Pl. 5, A and B.) This species is of special interest in this connection because it is one of the early winter annuals and probably serves along with (alfileria) *Erodium cicutarium* in harboring the curly-top virus over winter.

Among cultivated plants the tomato, *Lycopersicon esculentum* Mill, may now be included among the susceptible species. The effects of the disease on the tomato are rather of the mosaic type, a clearing of the veins of the younger leaves which gives these a slightly mottled appearance and a curling and distortion of the youngest leaves. Affected plants gradually wilt, turn yellow, and die within a few weeks after infection. The disease has not been found in commercial plantings, so far as is known to the writers. This is probably explained by the fact that the tomato seems to be unfavorable as a food plant for the leaf hopper. The variety known as Stone was used in the inoculation experiments.

The case of goose-foot, *Chenopodium murale*, is of special interest in considering the susceptibility of other plants. In the list previously published it was given as nonsusceptible. Before the time of that publication, nine experiments in regard to the susceptibility of *C. murale* had been performed. Viruliferous leafhoppers were caged on young plants in the attempt to produce the disease, or nymphs reared from viruliferous females on *C. murale* were tested on beets. The results of all these tests indicated that the species was not susceptible to the disease. The fact may be of some significance that all these early experiments were made at Spreckels, Calif., where the climate is usually cool. During the year 1919 and the two subsequent seasons, further tests were made at Riverside, Calif. In some instances the results were in part or entirely positive. In other words, *Eutettix tenella* nymphs reared on *C. murale* from viruliferous females produced curly-top in some or all of the plants to which they were transferred. Out of 43 experiments, however, only 13 gave positive results. The positive results show that under some conditions the plant harbors the virus of curly-top. In no case have clearly defined symptoms, such as swelling of the leaf veins or veinlet clearing, been noted, but in some of the plants a dwarfing of the later growth which may have been due to the disease has been observed. The fact that positive results were obtained in so small a proportion of the later tests and in none of

those done earlier shows that in its relation to this disease the species is peculiar. The climate of Riverside is in general warmer than that of Spreckels, particularly in summer, but opposed to the idea that climatic differences may explain the difference in results in the two localities is the fact that some of the positive results were secured during the winter. It seems probable that the species is resistant to curly-top.

An interesting observation made in connection with two of the experiments seems probably due to this seeming resistance. In two of the tests where nymphs from *Chenopodium murale*, the progeny of viruliferous leafhoppers, were caged on healthy young beets several of the beets soon developed faint clearing of the veins of some of the youngest leaves. The first symptom of curly-top is a clearing of the veins of the younger leaves. In these two tests, however, the vein clearing gradually disappeared in most of the plants and normal growth was continued. Only one or two plants developed typical cases of curly-top. One theory, which seems a plausible explanation, is that by passage through the *C. murale* the curly-top virus was so weakened or modified as to be rendered unable, in most cases, to effectively establish itself in the sugar beet. The point has not as yet been determined as to whether by means of nonviruliferous leafhoppers the virus may be obtained from those beet plants which show the faint vein clearing and subsequently recover.

As to variation in susceptibility to infection, the fact has been previously mentioned (*8*) that *Stellaria media* seems much more readily infected than the beet or buckwheat. There are some indications that *Erodium cicutarium* and *E. moschatum* and possibly *Calandrinia menziesii* are more readily infected than the beet. These three species, as well as spinach and tomato, die quickly as a result of the disease and in that sense are much more susceptible to the disease than several other species, e. g., *Malva parviflora*, cheese weed, and the sugar beet.

OVERWINTERING OF THE VIRUS

Few if any of those leafhoppers which leave the beet fields in the fall ever return. The observations by which this fact was established were in the main made in California. An observation made in Utah in the early summer of 1922 supports the belief that the same situation exists in that region. Near Elsinore, Utah, two sugar beet fields were inspected. In one of these fields a few cases of curly-top were found by diligent search, but certainly less than one-half of 1 per cent of the plants were affected. In the other field 1,200 plants were counted without finding any case of the disease. In both of these fields beet leafhoppers were present, and they had been there long enough to allow a considerable number of nymphs, their progeny, to emerge. It was estimated that there were 50 to 75 nymphs on each plant. Although the leafhoppers must have been on the beets for at least a month, there was practically no curly-top. Evidently most of the insects which flew into these fields in spring were nonviruliferous.

The question therefore arises as to where the new brood of insects which enters the fields in spring secures the curly-top virus. The suggestion has been previously made (*8*) that from susceptible annuals of the natural breeding areas some of these spring-brood leafhoppers may secure the virus. That this is not the only way in which the virus probably passes the winter should be made clear. As mentioned elsewhere in this paper, a small proportion of the leafhoppers usually remain in the cultivated area in the fall after the main body of the insects has departed. These "hold-over" insects, or "stragglers," being viruliferous, infect some of the early planted beets. Beets thus infected then serve as a source of virus for nonviruliferous insects that may come in later.

A third possible way in which the virus may be carried over winter is in old diseased plants growing in or near beet fields. In almost every old beet field such plants can be noted as making renewed growth in the spring. Frequently such plants are affected with curly-top. They would therefore serve as sources of the virus for any nonviruliferous insects that happened to feed on them.

CONTROL MEASURES

LEAFHOPPER CONTROL

The control of the beet leafhopper is naturally the first idea suggested for the prevention of curly-top damage, because this insect is the only known agency by which the disease is spread. The only feasible time of attack on the insects is in the early spring, when they first enter the beet fields and before they have deposited many eggs. They must then be killed or caught. The writers have tried the use of a hopperdozer. Large numbers of the insects were caught, but such a considerable proportion escaped that little or no benefit resulted. Liquid and dust sprays as insecticides and repellants have been tried to a limited extent by the writers. Kerosene emulsion in 6, 12, and 18 per cent strengths were tried. No significant decrease in the amount of curly-top resulted, even though some of the insects were killed. Nicotine sulphate in three different strengths gave only similar results. As repellants the following were tested: Bordeaux mixture, oil of citronella, naphthalene diluted with equal parts of lime, and mixtures of sulphur and tobacco dust with lime. In none of these tests was the benefit worthy of consideration. As has been mentioned before, the use of dusts, particularly mixtures containing nicotine sulphate, has been tried by others (*12*). The results thus far reported are not encouraging from the standpoint of commercial practice.

Control of the leafhopper by means of its natural enemies has been considered. There are three species of egg parasites and at least four parasites of the nymphs and adults. These parasites no doubt hold the leafhoppers in check to a large extent, but they can not be relied upon to prevent the production of curly-top in disastrous amounts.

TIME OF PLANTING

The control of *Eutettix tenella* by artificial means or by natural enemies seems at best a remote possibility, but by planting the beets early the damage due to this leafhopper can be avoided to a large extent. Emphasis should be placed in this connection on the fact that by early planting is not meant planting as early as possible in the fall, e. g., in October, but rather in the latter part of November and December, or as soon thereafter as practicable, and, for most of the affected regions, not later than February, if possible. Striking evidence in regard to the time-of-planting question was afforded by field observations in California in the season of 1918 at Corcoran and Bakersfield in the San Joaquin Valley and at Lancaster in the Mojave Desert. At Lancaster and Corcoran small plats of beets planted very early in the winter (October and November) were much more seriously diseased than were adjacent plats planted a month or two later. At Bakersfield, where the curly-top injury has usually been great, practically all of the planting of the season of 1917–18 was done in November, December, and January, and a good crop was produced, the beets being well advanced in size before the leafhoppers became abundant. At Corcoran, on the other hand, in the near vicinity of Lake Tulare, where the slow drying out of the soil retarded operations so that planting was delayed, as late as the latter part of March in some cases, great injury from curly-top resulted. In the vicinity

of Riverside and San Bernardino, in southern California, good crops have been produced by early planting (November and December), while the crops from late planting (March) in the same season were practically complete failures.

In regard to the time-of-planting question, from the standpoint of minimizing the injury from curly-top, two points must be given especial consideration. The plantings must not be made early enough in the fall to expose the young plants before the movement of the leafhoppers from the cultivated fields or late enough in the spring so that the beets will not have made a vigorous growth before the leafhoppers move into the cultivated areas. Mention should be made of the fact that usually some of the leafhoppers remain in the cultivated areas through the winter and that these stragglers may cause a considerable amount of damage. In general, however, the damage from those that remain in the cultivated areas, as compared with the damage when the main body of the insects is in cultivated districts, is relatively slight. With varying seasons the optimum time of planting will no doubt vary, but in general the safest plan is to plant as soon as possible after the middle of November and at least before the end of February. This recommendation is based primarily on conditions in California.

RESISTANT STRAINS

The fact that in almost every severely affected beet field a few individual plants will stand out conspicuously less affected than the rest has been noted by previous workers. The writers have repeatedly made this same observation. A project has been started by selecting the seemingly resistant plants from commercial fields, believing that by selection and possibly breeding a resistant strain satisfactory for commercial purposes may be developed.

Difficulties have been encountered in keeping over winter the beet roots which had to be removed from the field before harvest time. Siloing the beets in the ground, as is done in colder climates, has been found impracticable. Different methods of cold storage have been tried and a way of packing the roots for this purpose has been tested which seems, from the experience of one year, fairly satisfactory. Direct transplanting of the roots when harvested has been tried to a limited extent and found satisfactory.

Seed was produced from a few of the selected beets in the summer of 1921. Plantings of these strains together with control plats of commercial seed were tested for resistance in the autumn of that year. This test was made at Riverside, Calif., where leafhoppers were naturally abundant. In addition to the natural exposure, each plant of the selected strains was inoculated by caging on it a viruliferous leafhopper. The results of the test gave support to the belief that a strain of beets resistant to curly-top may be developed. Seeming differences in degree of resistance were noted between several of the selected strains and the commercial beets, and in one case this difference was marked (Pl. 5, C). Tests of these strains were made in the same manner but on a larger scale in the summer of 1922. The situation was complicated in this season by the fact that a large proportion of some of the apparently resistant strains developed seed stalks. On this account no satisfactory conclusions could be drawn as to resistance. Work is now in progress to determine the factors causing the premature seeding so that these factors may be eliminated.

More selections were made from commercial fields in California and Utah and also from the progeny test plats in the summer of 1922. The selection of more individuals from commercial fields and from the progeny plats will be continued and the progeny of these selections tested by inoculation for resistance to curly-top. The practice being followed in the growing of seed from the selected strains is to plant the mother beets singly or in small groups in private gardens, so that they will be isolated from each other.

SUMMARY

Curly-top disease of beets is characterized by a dwarfing of the whole plant, curling of the leaves, and irregular swelling of the veins on the under sides of the affected leaves. Marked phloem necrosis is produced throughout and shows as more or less conspicuous dark rings in cross sections of the root.

The disease is known to occur in only the semiarid regions of the western part of North America.

Curly-top is the cause of greater losses than any other sugar beet disease in this country. Very great damage to beets results when they are grown as an annual crop and when grown for seed production.

The exact nature of the cause of the disease is unknown, but the fact has been demonstrated that the virus can be transmitted by the beet leafhopper (*Eutettix tenella* Baker). No other means of transmission is known. The ability to produce the disease is not inherent in the leafhopper. There is some evidence that an interval of a few hours, an incubation period, must elapse after a nonviruliferous leafhopper has fed on a diseased plant before it can effectively transmit the virus to a healthy plant. Ten or more insects are more likely to produce the disease in an inoculated plant than when the inoculation is made with one or two insects. In cases where the disease is produced there is no difference in the incubation period or severity of the effect between inoculation with single insects and those with ten insects. A viruliferous leafhopper may not produce the disease each time it feeds on a healthy plant, even though the periods of feeding be 24 or 48 hours or even longer.

Tests in regard to the relation of light to infection indicate that plants kept in darkness during inoculation are more readily infected through the cotyledons than through the true leaves.

Tests of the effect of heat on the disease indicate that the virus is not destroyed by temperatures lower than those at which the beet tissue is killed.

Limited data suggest that the virus may be destroyed by desiccation.

The virus is distributed through all parts of an affected plant.

Limited data suggest evidence opposed to the claim that a specific bacterial organism is associated with curly-top.

The incubation period of the disease varies usually from 7 to 14 days. As short an incubation period as 4 days has occasionally been noted.

The incubation and development of the disease is retarded by low temperatures.

Very young plants are more readily infected than are older plants.

A wide range of species has been found susceptible to curly-top.

The virus probably overwinters in susceptible wild annuals, in volunteer beets, and in the insect.

No satisfactory control of the leafhopper by artificial or biological means has been discovered. Early planting usually avoids much of the injury. There is basis for belief that a resistant strain of beets may be developed.

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PLATE 1

Early stage of curly-top. Note the swelling of the veinlets on the lower surface of one leaf and the irregularity of the upper surfaces and the curled condition of the other young leaves.

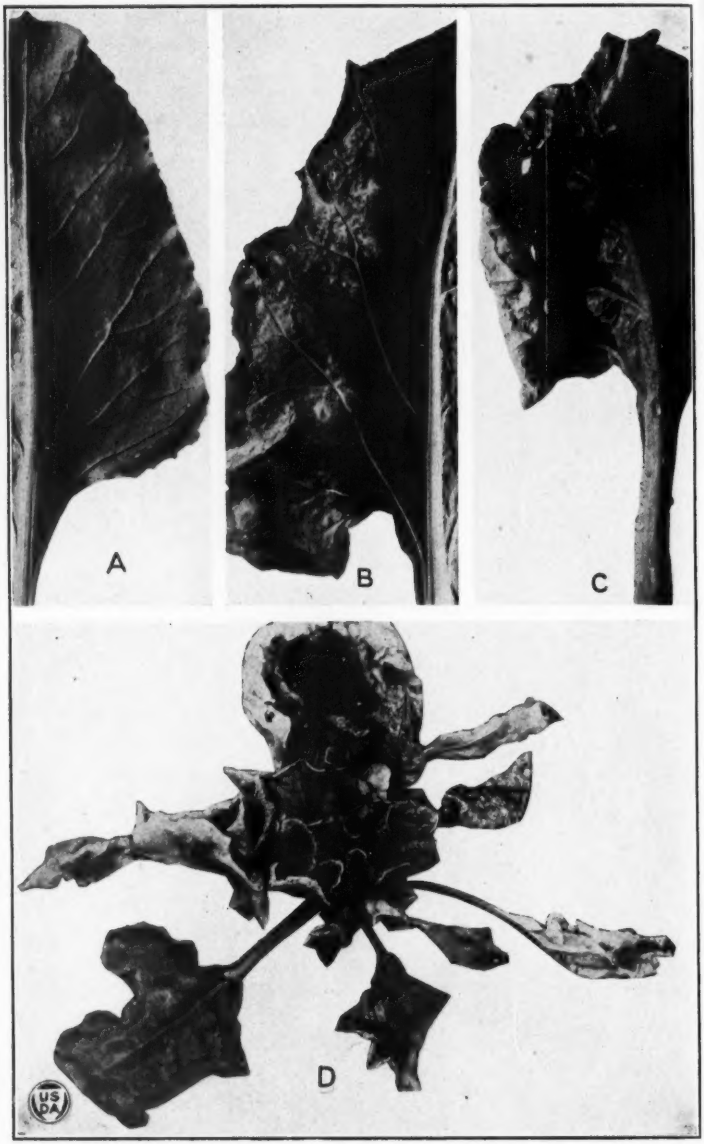

A
B
C
D

PLATE 2

A.—A type of curly-top beet leaf. Note the inconspicuous rounded swelling scattered over the surface.

B.—Curly-top beet leaf. Note the general distribution of the moderate, irregular swellings on the smaller veins. This specimen is representative of one type of response to the disease stimulus.

C.—Exudate on a curly-top leaf. The clear, viscid exudate has a sweet taste Fehling's test reveals the presence of sugars after hydrolysis with acid.

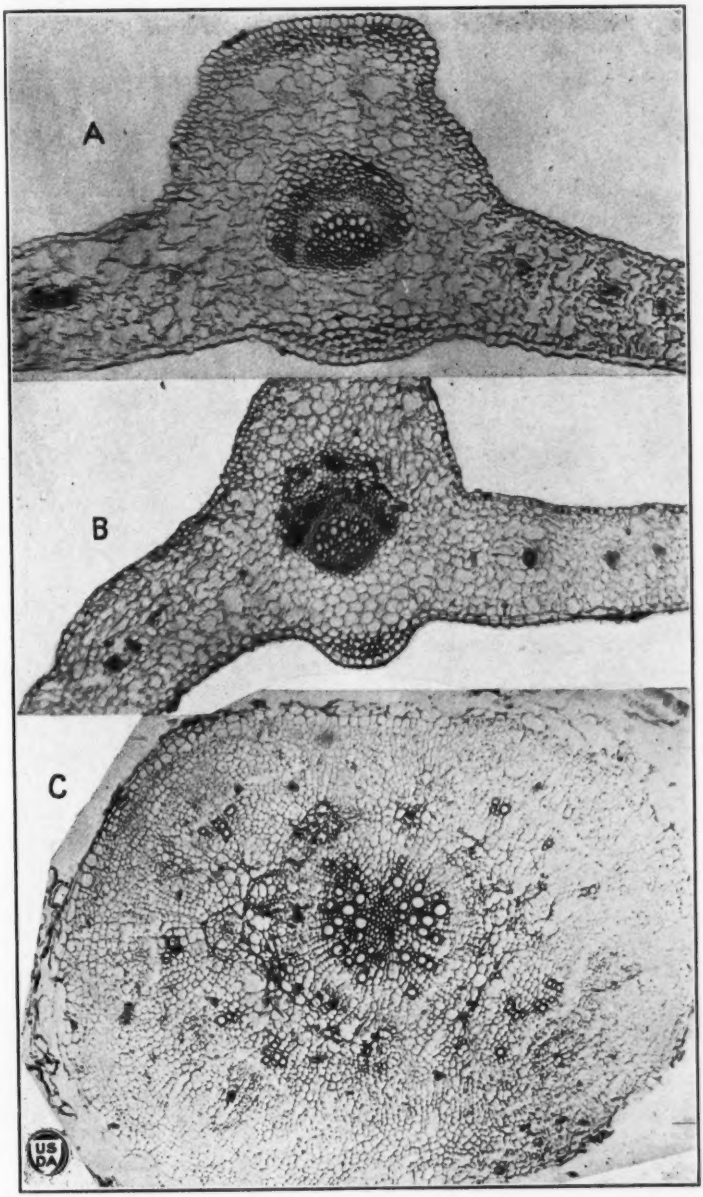
D.—Sugar beet curly-top. Plant inoculated on July 19, 1918, and photographed on August 29, 1918. Note the length of the old leaves, which were about mature when the plant was infected, in contrast with the dwarfed younger leaves.

PLATE 3

A.—Cross section of lateral vein of a healthy beet leaf.

B.—Cross section of lateral vein of a curly-top beet leaf. Note necrosis in the phloem region.

C.—Cross section of a young, curly-top beet root. Note the dark circle of necrotic phloem. All X about 70.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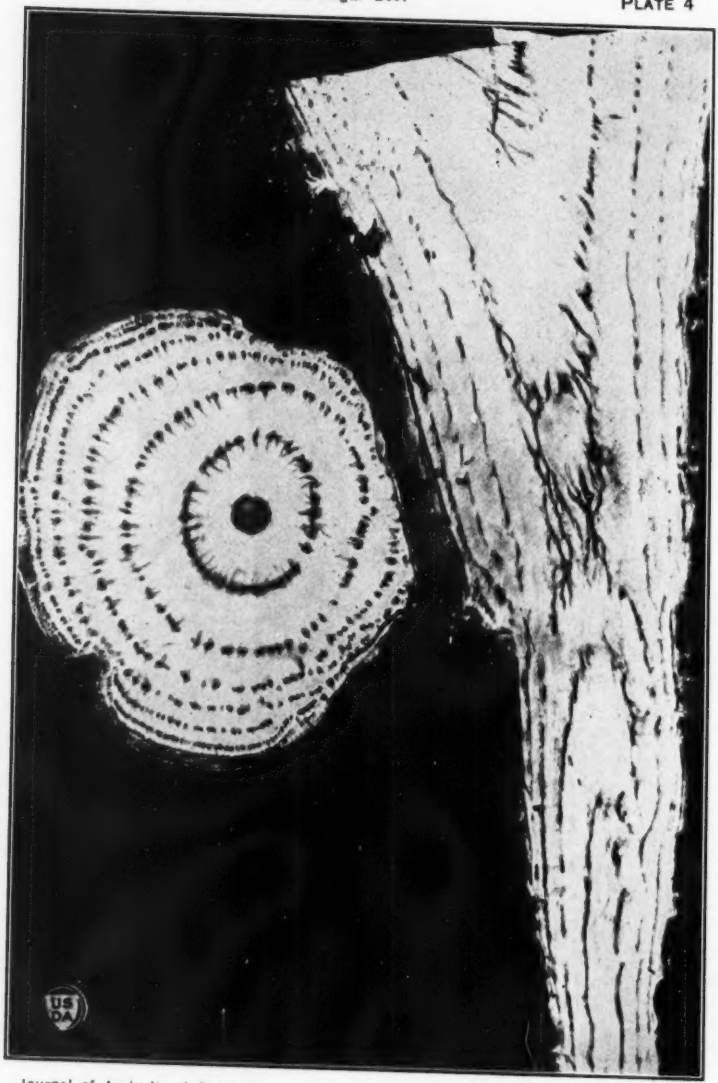


PLATE 4

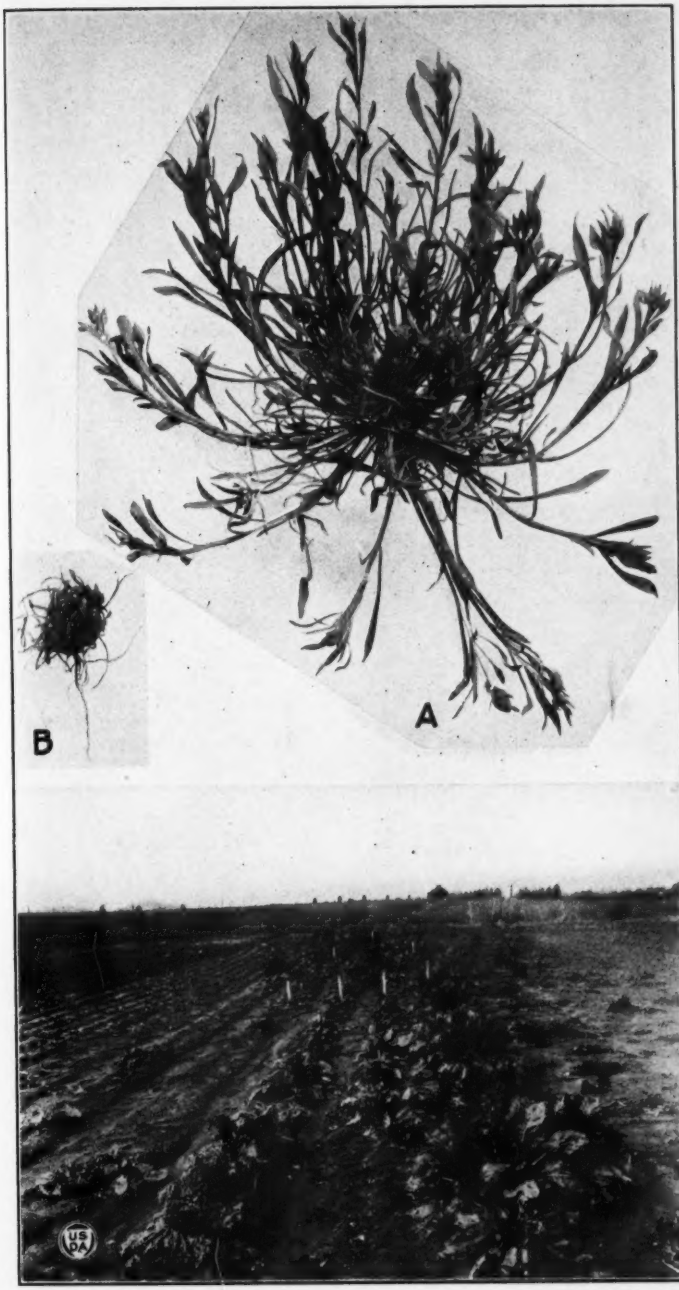
Sections of a curly-top beet root. The phloem necrosis which shows as the darkened tissues is often less conspicuous than in this specimen. X 2.

PLATE 5

A.—*Calandrinia menziesii* T. & C. Normal plant.

B.—Plant of about the same age but affected with curly-top. From plants diseased, as is *B*, from inoculation with viruliferous insects the disease has been communicated to healthy beets by the use of nonviruliferous leafhoppers.

C.—Resistance to curly-top. The beets in the row on the right were the progeny of a plant selected for resistance. The control row adjacent on the left was from commercial seed. Both rows were exposed to the same natural infestation of leafhoppers, and in addition all the plants of the selected strain had viruliferous leafhoppers caged on them.


A
B

PECAN SCAB WITH SPECIAL REFERENCE TO SOURCES OF THE EARLY SPRING INFECTIONS[1]

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INTRODUCTION

Owing to the extensive development of the cultivated pecan industry throughout the southeastern United States during the past 15 or 20 years, the economic importance of those diseases which materially decrease production has assumed a prominent position. Excluding rosette, which is apparently caused by, or at least associated with, unfavorable soil conditions, scab caused by *Fusicladium effusum* Wint., is preeminently the most important pecan disease.

DISTRIBUTION

Though the pecan is indigenous along the several rivers in Texas, the Mississippi, and its tributaries, as far north as central Illinois and southern Indiana, scab is known to exist only in the Southern States bordering the Atlantic Ocean and Gulf of Mexico, and in Arkansas. These facts indicate that high humidity as well as high temperature may be essential for its maximum development.

In the southeastern United States pecan scab was until recent years of serious economic importance only within a distance of 50 to 100 miles from the coast line, but now it seems to be extending its range of distribution inwardly. This condition is thought to be due largely to the increased number of plantings and to the frequent summer rains of the past few years, which have favored the rapid spread of the disease. The apparently rapid spread of scab in the central section of the southern pecan belt[2] may also be accounted for by the fact that the cultivated pecan industry is comparatively new and many orchards are young, having only recently come into bearing. A young orchard composed of one of the very susceptible varieties, such as Delmas, even when situated near the coast, may produce two or three crops of disease-free nuts before the scab fungus has become so well established as to reduce the yield of nuts. Even now the disease is of a less serious nature and fewer varieties are attacked 150 miles from the coasts than at points within 50 miles of them. Certain varieties, such as Van Deman, Schley, and Pabst, are very susceptible when grown near the Gulf and southern Atlantic coasts, but are at present only slightly attacked at points 100 miles from these coasts.

Not considering the natural agencies favoring the spread of pecan scab, the growers in many instances are partly, if not largely, responsible for the presence of the disease in its most accentuated form. This condition is brought about by the occasional neglect of orchards that have become unprofitable due to the loss of the crop by scab. Some growers think there will be a return of weather conditions, totally unfavorable for the development of the disease, which will permit their trees again to become productive. During this interval of neglect,

[1] Received for publication Feb. 27, 1924.

[2] The southern pecan belt is fairly well confined to that portion of the South in which cotton grows.

every leaf, stem, and nut growing on the susceptible trees becomes badly infected and the orchards become public nuisances, spreading the disease to near-by orchards in all directions.

The distribution of pecan scab is very noticeably associated with climatic conditions. With the exception of the humid Coastal Plain region, Texas ordinarily is practically free of the disease. Therefore, in that State, Delmas, one of the most susceptible varieties, is strongly recommended for planting. The disease is most destructive and attacks the widest range of varieties in the humid coastal region of Louisiana, Mississippi, Alabama, Georgia, and Florida.

VARIETAL SUSCEPTIBILITY

At present there is a wide variation in the degree of susceptibility of the different cultivated pecan varieties to scab. Some varieties are so severely attacked during seasons favorable for the dissemination and propagation of the fungus that the entire crop may be destroyed. Other varieties are so highly resistant that they may at present be considered almost immune. Wild or planted seedlings show the same variation in resistance.

It seems probable that, in some instances, the distribution of pecan scab and apparent differences in the relative susceptibility of the same varieties growing in different localities can be attributed to different strains of the pathogen. If different strains do not exist, then how can one explain why two susceptible varieties, as Delmas and Schley, can grow in contiguous rows for several years, the Delmas variety being badly infected but the Schley free of the disease? Then after an interval of a few years, the Schley also becomes infected, at first only slightly so, but later severely. Possibly, the Schley strain if there is such, was present in the Schley orchard during the time of its apparent absence, but increasing slowly, finally became abundant in the Schley orchard. It is possible that the strain on the variety Schley may have developed from the Delmas strain by some process of adaptation.

A classification of various cultivated varieties of pecans according to the degree of their susceptibility is not easy, in as much as the susceptibility of each variety varies in widely separated localities. What may be considered at present as a highly resistant variety in one locality may be a susceptible one in another place. Generally speaking, however, Delmas is the most susceptible variety followed by the varieties Georgia, Alley, Van Deman, Schley, Pabst, Mobile, Success and Moore, in the order named. The following varieties, while probably not entirely immune, are highly resistant: Teche, Curtis, Moneymaker, Russel, Stuart and Frotcher. The Texas varieties are purposely omitted from the list as ordinarily pecan scab is not an important disease in that State. Some Texas varieties, notably, the San Saba, are highly susceptible when planted in the humid region of the southeast United States.

DESCRIPTION OF THE DISEASE

ON THE LEAVES

On the leaves the infections first appear as elongated spots on the large vein on the underside of the pinnae or on the rachis and petiole, Plate 1, A. A few days later, spots may be noticed on the underside of the smaller veins also. The lesions on the small veins are at first elongated but later diffuse irregularly into the healthy tissues, forming more or less circular spots. Some infections appear to have taken place on the upper surface of the leaflets. Apparently not all spots begin on the veins, since some appear on the green parts lying within the network of the vascular system. The spots are at first olive brown in color, but turn almost black with age. They are quite conspicuous on the leaves and

especially so on the light yellow green of the young leaves. The infections on the leaf may be few or many, depending upon the variety, the abundance of sources of infection and the weather conditions. Leaves become more immune as they reach maturity and as a rule, are only slightly susceptible after they change from the light yellowish-green of the young stage to the later deep green color. The control of the disease would be much simplified if the pecan did not have the habit of putting out new leaves and twigs after the initial spring growth. A heavy second growth of foliage, as a rule, is produced about the 1st of July or soon after the usual summer rains commence. While on the light growth of new leaves during May and June new infections continue to occur thus forming new sources of infection, it is the new infections on the midsummer second growth of leaves which serve greatly to increase these sources of infection. A heavy increase of the disease on the nuts when they are about one-half grown as a rule follows the severe spotting of the second growth of foliage. Ordinarily, the development of the midsummer second growth of foliage extends over a period of four to six weeks. During this time new leaf surfaces are continually being exposed to infection. A protective spray may be applied today but to-morrow new leaflets are exposed and unprotected.

The damage done to the leaves as a whole is of minor importance, though considerable defoliation, especially in nurseries, sometime occurs (Pl. I, G), and heavily infected trees shed their leaves early in the autumn. Several infections on the veins of the ventral side of the leaflets cause them to curl downward as though injured by aphids. The punctures of aphids, often found feeding on the veins during early spring, are considered by Waite [1] as points of entrance for the pathogen. Possibly the greatest damage done to the leaves is due to the interference with their normal functioning caused by the partial girdling of the petioles from numerous infections near their bases.

ON THE NUTS

Narrow, elongated spots frequently appear first on the young nuts along the creases which run parallel to the long axis of the nut midway between the sutures of the pericarp, and which correspond morphologically to the veins of the leaflets (Pl. 1, C and D). Lesions are quite small at first and slowly enlarge, some attaining a diameter of about 12 mm. Most spots, however, cease to enlarge after they are about 3 mm. in diameter. The scab spots on the nuts may be either olive-brown or gray when first visible. Those infections which are at first gray later change to an olive-brown with a gray border. All scab spots, regardless of shape or color, possess a more or less jagged, irregular, and indefinite outline.

The greatest damage is done to the nuts, which may become undersized, faulty, or completely destroyed. The diseased areas are rather superficial, extending only slightly below the epidermis. The affected tissues become black, hardened, and somewhat cracked, but show no tendency to decay (Pl. 1, D). It is not uncommon, however, for secondary fungi to gain entrance through the ruptured surface caused by the scab pathogen and produce deep-seated lesions extending down through the exocarp to a depth of 2 to 2½ mm. The secondary fungi apparently do not enter the husk until the nut is from one-half to two-thirds grown. When scab infections occur while the nuts are small, or when numerous infections occur during midsummer, the growth and development of the nuts are completely retarded, and they either fall before attaining their normal size or blacken and hang on the trees all winter (Pl. 1, E; Pl. 2, D). The death of the nuts often seems to be due to the coincidence

[1] WAITE, M. B. PECAN SCAB [FUSICLADIUM EFFUSUM]. Science 33: 77-78. 1911.

of two factors: First a great number of infections directly causing the death of the nuts; and, second, infection of the pedicels, which are often so severely attacked that the flow of water and nutrient materials is partially or even completely shut off. Frequently the pedicel is killed outright while the nut is yet partly green.

ON THE TWIGS

The twigs of certain varieties of the pecan, especially the variety Delmas, are very susceptible to scab. The terminal twigs on old, neglected Delmas trees are often killed back from 7 to 15 cm. in one season by the scab pathogen (Pl. 2, A). The twigs of certain other pecan varieties, such as Alley, Van Deman, and Schley, are also susceptible but to a much lesser degree than those of the Delmas variety. Only the twigs of the current year's growth are attacked by the scab fungus, and these are susceptible only while they are young, growing rapidly, and before lignification takes place. The scab spots on the twigs are olive in color, while the fungus is sporulating, but are black after the spores have been washed away. In size, the lesions range from 0.5 to 3 mm. in diameter (Pl. 2, B and C).

ON THE CATKINS AND DORMANT BUDS

The writer has observed scab on pecan catkins only in a few instances. Apparently no injury is done to this floral part as the spots are few in number and have been seen only on the pedicels and bracts. The fungus does not seem to attack the pollen sacs.

It is not unusual to find from one to three scab spots holding over from the previous seasons on a dormant bud. From sections made of such infected buds, the writer is led to believe that mycelium does not extend deeper than the outer bud scale.

SOURCES OF PRIMARY INFECTIONS

According to those who have made a thorough study of the apple scab fungus, the conidial stage of which greatly resembles *Fusicladium effusum*, the principle source of early spring infections is the ascogenous stage formed within the tissues of the fallen leaves of the previous season. The writer has frequently observed several forms of ascomycetous fungi on dead pecan leaves, twigs, and nuts, but none has been specifically connected with the pecan scab fungus. While an ascogenous stage, which one would expect to be a Venturia, may exist, it is as yet unknown. Many orchard and laboratory observations have led the writer to believe that even should *Fusicladium effusum* produce a perfect stage it would certainly be only a contributory factor in the perpetuation of the fungus, as in the South the conidial stage regularly lives overwinter in great abundance.

TWIGS AS SOURCE OF PRIMARY INFECTIONS

Under certain favorable conditions, the hyphae in scab lesions ont wigs remains alive during the mild winters of southern Georgia, and the following spring becomes very important sources of the early spring infection. The mycelium does not remain alive overwinter in all twig lesions. The scab spots formed during the early part of the season apparently are not able to live after the cortex of the twig reaches a certain stage of maturity. By the end of the summer, such early formed lesions become flattened and cracked and the fungus within appears to be dead (Pl. 2, C and D). During the following spring many of them are either partially healed over or appear as black, sunken spots with slightly raised rings of cortical tissues surrounding them (Pl. 2, E). In these older spots, resulting from early spring infections, the fungus seldom lives through the winter and they do not, therefore, appear to serve as sources of the primary infections.

Twig lesions formed during the latter part of the summer or early autumn develop black more or less hemispherically shaped, sporulating stromata on their surfaces (Pl. 2, B). The author considers the sporulating stromata thus formed as very important sources of the early spring infections. They have frequently been observed producing conidia abundantly during April and May at the time the new leaves were unfolding and extremely susceptible to scab infection. Pecan twigs infected as illustrated in Plate 2, B, when collected at various times during the winter and spring months and placed in damp chambers for 48 hours, have almost invariably produced conidia from the stromata in great profusion. Spots on twigs that have been killed by a severe attack of scab during the previous season also serve to carry the disease over winter.

Primary infections from such sources as those just described are the most difficult to prevent. Pecan leaves are very susceptible to attack of the scab pathogen during the time they are unfolding. During this period, young pecan leaves are also very susceptible to spray injury, thus increasing the difficulty of preventing early leaf infections. Pecan leaves unfold over a period of several days, during which time an application of spray may do great damage to the foliage and serves only to protect those pinnae already unfolded and exposed.

OLD NUTS AS SOURCE OF PRIMARY INFECTIONS

Nuts that have been killed by the scab fungus during the previous summer are, as a rule, important sources of the early spring infections. All such nuts however, seem not to carry the fungus through the winter, but only those that have attained one-half size or more before being killed by the disease. The nut, illustrated in Plate I, E, is a good example of this type of the primary infection The dark blotches, seen on the stem-end of such nuts will produce conidia during the early spring weeks. Frequently, nuts are slightly spotted with scab during late summer. Infections taking place at this time may cause no damage to the nuts since the kernel develops and matures normally. These late infections usually form the sporulating stroma and serve as a means of carrying the disease through the winter. Either empty shucks or late season infected nuts are quite noticeable as sources of the early infection when they remain on the trees over winter and become the center of a number of scab-infected leaves the following spring.

LEAVES AS SOURCE OF PRIMARY INFECTIONS

Scab spots that have formed on the smaller veins of the leaflets or those that have formed within the network of the vascular system do not form sporulating bodies and are, therefore, not believed to harbor the fungus through the winter. Lesions on the mid-veins of the pinnae, rachises and petioles, especially those that form near the base of the petioles, produce the hold-over stroma, and consequently serve as sources of the primary infections.

CONTROL MEASURES

During the pioneer days of the cultivated pecan industry, the variety Georgia was thought to be the most susceptible to pecan scab. The growers early realized the seriousness of the disease on this variety and promptly top-worked the Georgia to varieties considered at that time as being resistant or immune. Mistakes were made during this period of varietal changing, owing to the then apparent resistance of some varieties now known to be exceedingly susceptible. For instance, the Georgia variety was top-worked to Delmas. Later, Delmas orchards were top-worked to the variety Schley. The last-named variety is now known to be very susceptible in some localities. While many growers are still discarding the susceptible varieties by top-working to the more resistant ones, this method

of disposing of the disease is less popular than formerly, owing to the apparent adaptation of the fungus to the more resistant ones. Spraying is now being considered somewhat more seriously as a means of reducing the loss by scab, but as yet most of the growers seem to prefer top-working to the more painstaking and labor-involving operation of spraying.

During the past two years the writer, in search of the most satisfactory spray for pecan-scab control, has been devoting considerable attention to trials with different kinds of fungicides commonly used in apple and peach orchards. In the order named, Waite,[4] Spooner,[5] McMurran and Demaree,[6] and Neal[7] have shown Bordeaux mixture to be quite effective as a spray for the control of pecan scab. Because this spray sometimes causes serious injury to the foliage an attempt was made to find some spray that would be effective and yet cause no serious foliage injury. The following fungicides have been used: Sulphur dust, Bordeaux dust, lime sulphur solution, wettable sulphur, self-boiled lime sulphur, lime sulphur modified by the addition of 1 pound of copper sulphate to each 50 gallons of spray and self-boiled lime sulphur modified by the addition of 2 pounds of copper sulphate to each 50 gallons of spray. Both types of dust, wettable sulphur and self-boiled lime sulphur, even when modified with copper sulphate, proved to be ineffective under the conditions tried. Lime sulphur solution when used at the strength of 1 gallon of the concentrated material (32 to 33° B.) to 50 gallons of water, controlled the disease quite satisfactorily during the season of 1922. This spray, however, proved to be much less effective during the rainy summer of 1923. From the results of the work so far conducted, it appears that the choice of a spray will be limited to Bordeaux mixture which is ordinarily effective but may cause serious foliage injury under certain weather conditions, and lime sulphur solution, a spray only slightly injurious to the foliage, but whose capabilities as a preventive of pecan scab are not yet fully demonstrated.

Since it is difficult to control pecan scab even under the most favorable conditions, advantage should be taken of every possible opportunity to reduce in number the sources of infection. As previously mentioned, the unfolding leaves are quite susceptible to spray injury. The foliage is also very susceptible to scab infection during this same period. Therefore, the importance of destroying in so far as possible all the sources of the primary infection is quite evident. The writer has been informed of instances where fire swept through badly infected orchards, burning the débris on the surface of the ground. As a result, it was reported that there was a very pronounced reduction in the amount of the disease in these orchards during the season following. The effect of numerous overwintering infection sources on control by spraying was brought to the writer's attention in a very striking manner last season. An orchard of the variety Schley, at Quincy, Fla., was sprayed on the following dates: April 10, May 9, May 23, June 13, July 5, and 27, 1923. On account of frequent rains during June and July, the spray could not be applied according to any prearranged schedule. No attempt was made to destroy the sources of the primary infections. The soil had not been plowed and the last year's leaves, shucks and mummied nuts were left upon the ground. Consequently, a severe primary infection of the leaves took place very early in the spring, even before it was

[4] WAITE, M. B. NUT DISEASES WITH SPECIAL REFERENCE TO THE PECAN. Proc. Amer. Pomol. Soc. 32: 182-190. 1911.

[5] SPOONER, C. S. PECAN SCAB (FUSICLADIUM EFFUSUM). Georgia State Bd. Ent. Bul. 49: 38-43, illus. 1918.

[6] MCMURRAN, S. M., and DEMAREE, J. B. DISEASES OF SOUTHERN PECANS. U. S. Dept. Agr. Farmers Bul. 1129, 22 p., illus. 1920.

[7] NEAL, D. C. SPRAYING EXPERIMENTS FOR THE CONTROL OF PECAN SCAB IN MISSISSIPPI. Miss. Agr Exp. Sta. Bul. 208, 14 p., illus. 1921.

thought to be safe to spray on account of probable injury to the tender foliage. The result of this spraying experiment was a complete failure as far as control was concerned. In spite of six applications of spray, of which five were with standard strength Bordeaux mixture, the disease completely destroyed the crop. This orchard was sprayed four times with Bordeaux mixture during the previous season and it is thought that twig infections of that year played a very minor part in causing the primaty infections of the past year.

As a contrast to the Quincy, Fla., experiment, the results are given below of a spraying test at Thomasville, Ga., on the same variety. This orchard had been sprayed for two years previous, and the ground was carefully plowed during late winter so that all leaves, petioles, and shucks were buried under the soil. The disease did not appear in this orchard until about the first of June. An application of spray at this time was highly desirable but was postponed until June 6 on account of rainy weather. The second application was made July 3, and the third and last one August 3. As a result of this work, approximately 89.6 per cent of the crop of the Bordeaux-sprayed trees was saved. The control trees in this orchard matured some good nuts, but 56 per cent of the crop fell before maturity. Of those nuts remaining on the trees until harvest 20 per cent were either totally bad or faulty. The sprayed nuts averaged 64 to the pound, while the unsprayed ones averaged 74. It is not thought that the weather conditions at Thomasville were more favorable for a spraying experiment than at Quincy, Fla., as it was unusually rainy and cloudy during the season. Including days with a trace, rain fell on 19 days in May, 24 days in June, 21 days in July, and 15 days in August at Thomasville. No record of the rainfall at Quincy, Fla., is available.

A standard spray schedule for pecan scab has not yet been definitely established. A spray schedule that produces satisfactory results one season often proves to be entirely ineffective another one. Very satisfactory results were procured at Dewitt, Ga., in 1922 by applying the spray on the following dates: May 1, May 17, June 6, June 28, July 19, and August 19. A similar schedule followed during the season of 1923 in the same orchard did not control the disease satisfactorily. At Baconton, Ga., in 1922, excellent results were obtained by applying spray on the following dates: June 13, July 6, July 27, and August 23. In 1923, despite the fact that spraying in this orchard was begun a month earlier and two more applications were made than in 1922 the results were not satisfactory.

As a general recommendation in the light of our present knowledge and experience, the first application of a protective spray should be made immediately after the nuts have set. Later applications should be made at intervals of two or three weeks. The weather conditions, the amount of scab in the orchard the year previous, the relative susceptibility of different varieties, and the attention paid to sanitation are factors that must govern the number and time of application.

DORMANT SPRAY

In an endeavor to eliminate the twig lesions as sources of the early spring infections, Bordeaux mixture containing 8 pounds of bluestone and 8 pounds of stone lime to 50 gallons of water and lime sulphur solution prepared by adding 6 gallons of the concentrated solution (32 to 33° B.) to 42 gallons of water have for three successive years been applied as dormant sprays. The time of application was in all cases delayed until a few days before or even after the buds begin to swell. While it appears that a strong fungicide would kill the hold-over stromata, only slight evidence has been secured by the writer favorable to the use of the winter spray.

DIFFICULTIES ENCOUNTERED IN ENDEAVORS TO CONTROL PECAN SCAB

Those attempting to control pecan scab encounter several perplexing difficulties. In addition to the problems arising in the control of other deciduous fruit diseases, even more perplexing ones appear. The problem of spraying the tops and centers of some of the larger pecan trees is not an easy one. Despite the youthfulness of the cultivated pecan industry, some of the older cultivated trees have attained the height of 50 to 60 feet with a spread of 40 to 60 feet. The usual tower and spray rods are not practicable to use when spraying trees of such dimensions and resort must be had to the high-power spray outfits and spray guns. Most of the spray guns used by the writer are not capable of carrying the desired misty spray to the tops of the taller trees. It is hoped that horticulturists will devise some system of pruning that will tend to reduce the height of the trees and open up their centers so as to facilitate spraying operations.

The pecan nut is susceptible to the attack of the scab pathogen from the time it is first formed, which is ordinarily during the latter part of April or the first part of May, until it has completed its growth. Any meristematic tissue seems to be subject to infection, necessitating, in case of the very susceptible varieties, frequent applications of spray from the first of May to the middle of August.

Another difficulty in controlling pecan scab is due to the susceptibility of certain varieties such as Delmas to twig infection. Since there is ample evidence that large numbers of spores are produced on infected twigs in the early spring, one can readily see how easily the unfolding leaves situated in such close proximity to these sources of infection may become infected. Twig infections on the current year's growth also serve to propagate the fungus during the summer.

The rainy season through the central and southeastern sections of the pecan belt usually begins about the middle of June, and normally extends over a period of approximately three months. The average number of rainy days for June, July, and August at Thomasville, Ga., according to the United States Weather Bureau is 12, 15, and 14, respectively. Then, normally, rain may be expected on an average of almost every other day during those three months. Occasionally the number of rainy days greatly exceeds the average, as for example, July, 1921, had 27; July, 1922, 18; June, 1923, 24; and July, 1923, 21.[8] It is often difficult and occasionally impossible, on account of boggy soil, to apply spray at the times one thinks it should be put on during June, July, and August in the Coastal Plain region.

Coincident with the rainy period, the pecan trees put on a new growth of twigs and foliage, which greatly complicates spraying operations on account of the extreme susceptibility of the new foliage.

SUMMARY

Excluding rosette, pecan scab, caused by *Fusicladium effusum* Wint., is the most important disease affecting the pecan.

The disease is known to exist in all the Southern States bordering the Atlantic Ocean and Gulf of Mexico and in Arkansas.

At the present time there is known to be a wide variation in the degree of susceptibility of the different cultivated pecan varieties to the scab disease. Some varieties are very susceptible while others are almost immune.

The disease is known to attack the nuts, twigs, leaves, dormant buds, and catkins. Very little, if any, damage is done to the buds and catkins. Consider-

[8] The Weather Bureau indicates a rain measuring less than 0.01 of an inch as a trace. Days having only a trace of rain are included in the number of rainy days herein reported. The author feels justified in including the days when the precipitation indicates only a trace, as a misty, cloudy day, when the actual precipitation measures less than 0.01 of an inch may be much more favorable to spore germination and infection than a heavy shower followed by bright sunshine.

able defoliation, especially in nurseries, sometimes occurs. Severely infected trees usually shed their leaves earlier during the fall than they normally should. Occasionally the tender tips of the twigs are killed back from 5 to 15 cm. The greatest damage is done to the nuts which may become undersized, faulty, or rendered entirely worthless.

An ascogenous stage of the pecan scab fungus is not known to exist. The fungus lives through the winter months in the form of more or less hemispherically shaped stromata situated in lesions upon the surface of twigs, rachises, petioles, and shucks. These stromata produce conidia the following spring.

Hygienic measures tending to eliminate, as completely as possible, the various sources of the early spring infections, are very important as a means of keeping the disease within bounds so that spraying may be made effective. Twig lesions can be largely prevented by thorough summer spraying. The most practical method of disposing of the infected leaves and shucks after they fall to the ground is the thorough plowing under of these parts during late winter. If practicable to do so, the raking and burning of these affected parts would also be effective.

Of the various available spray materials tried Bordeaux mixture was the most effective, although it sometimes causes serious injury to the foliage.

Strong Bordeaux mixture and strong lime sulphur solution used as dormant sprays did not perceptibly reduce the amount of infection.

The following are mentioned as instrumental in making effective control of pecan scab difficult: The large size of the pecan trees to be sprayed; the normal frequency of summer rains prevailing in the southern pecan belt during June, July, and August; the long season of susceptibility of the pecan nut to scab infection; the close proximity of the young leaves to hold-over stromata on the twigs; and production of a second growth of leaves and twigs, subject to infection if not protected by spray.

PLATE 1

A.—Pecan leaf showing various types of infection of pecan scab. The photograph was taken especially to show the elongated lesions of the midrib of the pinnae.

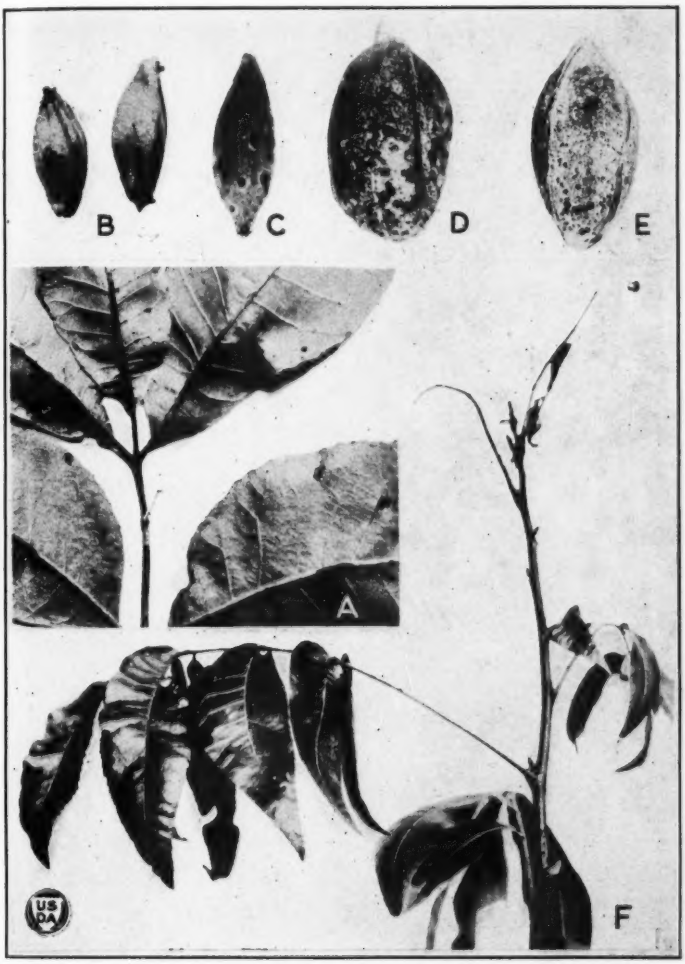
B.—Young pecan nuts showing elongated lesions situated lengthwise along the center of the segments of each nut. About natural size.

C.—Pecan scab lesions as they appear irregularly over the surface of the nut. About natural size.

D.—A pecan nut about one-half developed, showing very numerous scab infections. Three-fourths natural size.

E.—A pecan nut killed during midsummer, due to a heavy infection of pecan scab. The dark blotches on the stem-end of the nut are hold-over stromata Such nuts usually fall to the ground before harvest, but some remain attached to the twigs all winter.

F.—A pecan twig taken from a nursery showing premature defoliation due to pecan scab. Photograph taken July 17, 1920.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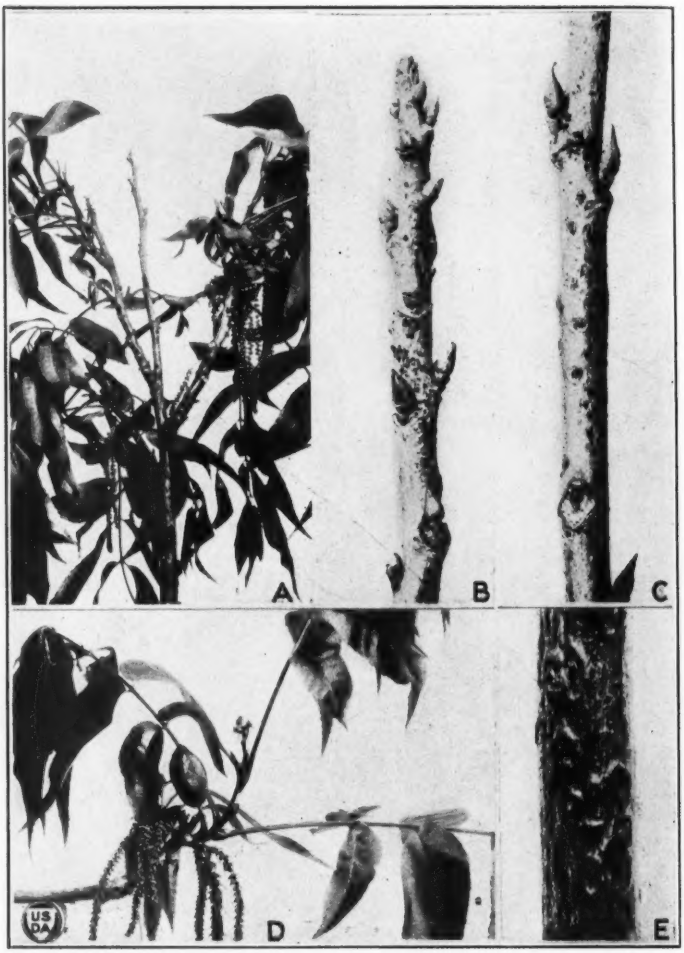
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PLATE 2

A.—A pecan twig killed during the year previous by a severe infection of pecan scab.

B.—The black spots on the middle portion of the twigs are slightly raised masses of closely woven mycelium formed by the pecan scab fungus. Such spots produce Fusicladium spores during early spring. About natural size.

C.—The large flat lesions on the middle portion of the twig are the results of early spring infections. The fungus is dead in such spots and does not form the overwintering stromata. Natural size.

D.—A last year's scab-killed nut, situated among the new leaves and inflorescence. Scab-killed nuts are often a fertile source of the early spring infections.

E.—A pecan twig showing old scab spots that are now beginning to heal over.

ANTHELMINTIC EFFICIENCY OF CARBON TETRACHLORID IN THE TREATMENT OF FOXES[1]

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Investigations conducted by the Bureau of Biological Survey of the United States Department of Agriculture tend to indicate that many foxes, both in the wild and on ranches, are infested with hookworms, principally *Uncinaria stenocephala* and occasionally *Ancylostoma caninum*. Although many foxes harboring hookworms manifest no apparent symptoms of infestation, observations made indicate that heavy infestations may be attended with impaired health and may even result in death.

In view of the fact that Hall (*2*)[3] found chemically pure carbon tetrachlorid in sufficient doses to be relatively effective and safe in the treatment of dogs for the removal of hookworms, it was considered advisable to test this drug as an anthelmintic for foxes. The results of the experiments conducted are reported in this paper.

ANTHELMINTIC EXPERIMENTS

In the experiments on foxes, the technic was similar to that used by Hall alone or in collaboration with other investigators (*2*, *3*). Chemically pure carbon tetrachlorid was given in hard gelatine capsules or in soft elastic globules to foxes that had been fasted from 16 to 20 hours. The only animals which were not fasted the usual period were foxes No. 792 and 793, two 1-month-old nursing pups. No purgative was given before or after the administration of the dose, except to fox No. 88.

After treatment each animal was confined in a small cage which was provided with a heavy gauge 1-inch woven-wire bottom and set on supports, with a large galvanized iron collecting pan underneath to catch all feces and worms passed. In the use of cages of this type it was found that the number of worms eaten or adhering to the feet or fur of the foxes was reduced to a minimum. The feces and worms passed were collected daily, screened, and all worms passed were identified and counted.

From three to five days after treatment the animals were killed with either a subcutaneous or an intrathoracic injection of strychnin sulphate solution. A post-mortem examination was made of each animal to determine the kind and number of worms remaining in the alimentary tract; also to make note of the gross pathological changes, especially those apparently due to the drug. The percentage efficacy of the treatment in all cases was the figure obtained by ascertaining the relation of the number of worms passed to the combined total of the number passed after treatment and the number found on post-mortem.

[1] Received for publication February 25, 1924

[2] Dr. H. L. Van Volkenberg resigned March 31, 1923, to work under the Bureau of Fisheries on the diseases and parasites of blue foxes on the Pribilof Islands, Alaska.

[3] Reference is made by number (italic) to "Literature cited," . 337.

A dose rate of 0.1 cc. per kilo was given in hard gelatine capsules to the following:

Fox No. 201.—Weight 5.24 kilos; passed no worms. Post-mortem fourth day, 12 hookworms, 2 ascarids, and 1 tapeworm; 0 per cent vs. hookworms, ascarids, and tapeworms. No apparent symptoms or gross lesions due to the treatment.

Fox No. 209.—Weight 2.5 kilos; 19 hookworms first day, 3 hookworms second day; retained no worms; 100 per cent vs. hookworms. Post-mortem fourth day; liver slightly swollen, with tissues bulging slightly on section; region of congestion between cortex and medulla of kidneys; slight congestion of mucosa of small intestine.

A dose rate of 0.2 cc. per kilo was given in hard gelatine capsules to the following:

Fox No. 203.—Weight 5.45 kilos; passed 18 hookworms first day; retained no worms; 100 per cent vs. hookworms; no apparent symptoms or gross lesions.

Fox No. 204.—Weight 6.36 kilos; passed 7 hookworms first day. Post-mortem fourth day; 1 hookworm and 2 ascarids; 88 per cent vs. hookworms and 0 per cent vs. ascarids; no apparent symptoms or macroscopic lesions due to the treatment.

Fox No. 786.—Weight 4.1 kilos; 4 hookworms and 21 ascarids first day; 3 hookworms second day; 2 ascarids third day; killed fourth day and 1 ascarid found; 100 per cent vs. hookworms and 92 per cent vs. ascarids. No apparent symptoms or gross lesions.

Fox No. 787.—Weight 3.4 kilos; 11 hookworms first day; 1 hookworm second day; retained no worms; 100 per cent vs. hookworms; no apparent symptoms. Post-mortem fourth day; slight congestion of mucosa of duodenum; liver darker in color than normal, with tissues friable and bulging slightly on section; spleen swollen.

Fox No. 788.—Weight 3.2 kilos; one ascarid first day; killed fourth day and no worms recovered. Post-mortem: Slight congestion of the mucosa of the posterior region of the small intestine; liver light in color and tissues friable; spleen dark and swollen.

A dose rate of 0.25 cc. per kilo was given in soft elastic globules to the following:

Fox No. 332.—Weight 4.3 kilos; one hookworm first day; four hookworms second day; killed third day and no worms retained; 100 per cent vs. hookworms; no apparent symptoms. Post-mortem: Tissues of kidney lighter in color than normal and bulging slightly on section; liver slightly darker in color than normal; mucosa of stomach and duodenum congested; wall of duodenum thickened.

Fox No. 334.—Weight 4.1 kilos; 3 ascarids and 8 intestinal flukes first day; 1 hookworm second day; killed third day and 2 hookworms present; 33 per cent vs. hookworms and 100 per cent vs. ascarids and intestinal flukes. No apparent symptoms or gross lesions due to the drug.

Fox No. 356.—Weight 1.86 kilos; age 3 months; 43 hookworms and 24 ascarids first day; 7 hookworms and 1 ascarid second day; killed third day and 3 hookworms and 1 ascarid recovered; 94.3 per cent vs. hookworms and 96.1 per cent vs. ascarids. Post-mortem: Liver greatly enlarged and darker in color than normal; tissues of kidney bulged slightly on section; region of congestion between cortex and medulla of kidney; mucosa of duodenum slightly congested; wall of duodenum thickened.

A dose rate of 0.3 cc. per kilo was given either in soft elastic globules or hard gelatine capsules to the following:

Fox No. 88.—Weight 2.38 kilos; age, 3¼ months; subject to a disease characterized by convulsions; dose given in hard gelatine capsules and followed in 4 hours by 5 cc. of castor oil in capsules; 290 hookworms (*280 Uncinaria stenocephala* and 10 *Ancylostoma caninum*) first day: Fifth day convulsions reappeared and animal died; retained three hookworms; 99 per cent vs. hookworms. Post-mortem: Liver and kidneys congested; brain edematous; petechiae and ecchymosis of small intestine; ecchymotic and suggulation hemorrhages of the cecum and colon; doubtful if carbon tetrachlorid was a factor in the cause of death.

Fox No. 197.—Weight 3.4 kilos; drug in hard gelatine capsule; passed 13 hookworms first day; killed fourth day and no worms present; no apparent symptoms. Post-mortem: Liver slightly enlarged; mucosa of stomach slightly congested; few small scattered foci of necrosis of the mucosa of stomach; mucosa of small intestine slightly congested.

Fox No. 198.—Weight 3.63 kilos; drug in hard gelatine capsule; passed 13 hookworms and 1 ascarid first day; killed fourth day and no worms present; 100 per cent vs. hookworms and ascarids; no apparent symptoms or gross lesions due to treatment.

Fox No. 200.—Weight 2.7 kilos; drug in hard gelatine capsule; no worms found in feces or upon autopsy examination; no conclusions as to efficacy; no apparent symptoms. Post-mortem fourth day: Slight congestion of the kidneys and mucosa of duodenum.

Fox No. 333.—Weight 3.3 kilos; drug in soft elastic globule; passed two hookworms and two intestinal flukes first day; killed third day and no worms present; 100 per cent vs. hookworms and intestinal flukes; no apparent symptoms; mucosa of stomach and small intestine slightly congested.

Fox No. 335.—Weight 3.1 kilos; drug in soft elastic globule; passed two hookworms first day; no worms present when killed third day; 100 per cent vs. hookworms; no apparent symptoms. Post-mortem: Liver darker in color than normal and blood oozed on section; tissues of kidney bulged slightly on section; mucosa of duodenum congested and wall thickened; Peyer's patches prominent.

Fox No. 367.—Weight 1.7 kilo; age 3 months; drug in soft elastic globule; 10 hookworms and 15 ascarids first day; 1 ascarid second day; killed third day and 4 hookworms found; 71.4 per cent vs. hookworms and 100 per cent vs. ascarids; no apparent symptoms. Post-mortem: Liver icteric and light in color with tissues friable; mucosa of small intestine slightly congested.

Fox No. 792.—Weight 0.68 kilo; age 34 days; length of fast not known, because this unweaned pup was removed from vixen and treated 1 hour later; drug in soft elastic globule; passed 15 ascarids first day; died second day after treatment; no worms present on autopsy; 100 per cent vs. ascarids, symptoms of distress, lassitude, weakness, anorexia, and marked constipation became apparent 20 hours after treatment. Constipated feces were reddish in color. Post-mortem: Liver light in color and icteric; lobules of liver distinctly visible, being yellow at periphery and a bright red at center; suggulation hemorrhages of the endocardium of left ventricle; pericardium contained an excess of fluid; mesenteric lymph glands enlarged and edematous, inflammation of the mucosa of jejunum and ileum, contents of large intestine and posterior region of small intestine constipated and hemorrhagic.

Fox No. 793.—Weight 0.8 kilo; age 35 days; length of fast not known because this unweaned pup was removed from its vixen and treated 1 hour later; drug given in soft elastic globule; passed 14 ascarids first day; died two days after treatment and 1 ascarid recovered; 93 per cent vs. ascarids; constipation, distress, lassitude, and anorexia became apparent day after treatment; feces passed with worms were hemorrhagic. Post-mortem: Suggulation hemorrhages of the endocardium of the left ventricle; ecchymotic hemorrhages of the lungs; liver light in color and icteric; lobules of liver distinctly visible, being yellow at the periphery and bright red at the center; stomach catarrhal; small intestine inflamed, with evidence of hemorrhage into lumen of posterior region; contents of cecum and colon constipated and hemorrhagic.

A dose rate of 0.35 cc. per kilo was given in soft elastic globules to the following:

Fox No. 348.—Weight 2.86 kilos; 17 hookworms first day; killed second day and no worms retained; 100 per cent vs. ascarids; no apparent symptoms or gross lesions due to the drug.

Fox No. 355.—Weight 1.4 kilos; age 3 months; 74 hookworms and 36 ascarids first day; 1 ascarid third day; killed fourth day; 1 ascarid retained; 100 per cent vs. hookworms and 97 per cent vs. ascarids; no apparent symptoms. Post mortem: Liver light in color and icteric, with tissues friable; pericardium contained a slight excess of fluid; mucosa of duodenum slightly congested.

A dose rate of 0.4 cc. per kilo was given in soft elastic globules to the following:

Fox No. 728.—Weight 4.54 kilos; affected with sarcoptic mange, but in good physical condition otherwise; 12 hookworms first day; killed fourth day; no worms retained; 100 per cent vs. hookworms; no apparent symptoms. Post-mortem: Mucosa of stomach and small intestine slightly congested; wall of small intestine thickened; region of congestion between cortex and medulla of kidneys.

Fox No. 731.—Weight 4.1 kilos; no feces passed in first two days; one hookworm and two ascarids third day; killed fourth day and no intestinal worms present; 100 per cent vs. hookworms and ascarids; no apparent symptoms. Post-mortem: Congestion of liver and pancreas; kidneys darker in color than normal, with region of congestion between cortex and medulla; mucosa of small intestine congested; considerable mucus and fibrin adhered to mucosa of small intestines; heart muscle pale.

A dose rate of 0.45 cc. per kilo was given in soft elastic globules to the following:

Fox No. 729.—Weight 3.64 kilos; affected with sarcoptic mange, but apparently in good physical condition otherwise; 18 hookworms first day; 8 hookworms second day; killed third day and 1 hookworm found; 96 per cent vs. hookworms; no apparent symptoms. Post-mortem: Mucosa of stomach and small intestine slightly congested, kidneys darker in color than normal, with region of congestion between cortex and medulla.

Fox No. 730.—Weight 3.7 kilos; affected with sarcoptic mange, but apparently in good condition otherwise; 169 hookworms, 1 ascarid, and 2 immature Physaloptera first day; killed third day, and no worms retained; 100 per cent vs. hookworms and ascarids; and probably 100 per cent vs. Physaloptera; no apparent symptoms. Post-mortem: Liver and kidneys darker in color than normal; region of congestion between cortex and medulla of kidney; wall of small intestine thicker than normal.

A dose rate of 0.5 cc. per kilo was given in soft elastic globules to the following:

Fox No. 325.—Weight 3.8 kilos; four hookworms and one ascarid first day; killed fifth day and no worms found; 100 per cent vs. hookworms and ascarids; no apparent symptoms. Post-mortem: Liver darker in color than normal and swollen, with blood oozing on section; slight congestion of mucosa of small intestine.

Fox No. 500.—Weight 3 kilos; age 4 months; 31 hookworms and 70 ascarids first day; retained 2 ascarids; 100 per cent vs. hookworms and 97 per cent vs. ascarids. Animal became constipated and died 30 hours after treatment. Weakness and restlessness became apparent an hour or so before death. Post-mortem findings were: Abdominal cavity filled with dark unclotted blood, which turned bright red upon

exposure to air; this blood failed to clot upon standing over 24 hours; suggulation hemorrhages of the endocardium of the left ventricle, the lungs were pale and anemic with a few foci of ecchymosis present; the liver was icteric and its tissues friable; lobules of liver distinctly visible, being bright red at the center and yellow at the periphery; pancreas congested; tissues of the mesenteric lymph glands were hemorrhagic; the mesentery and diaphragm showed suggulation hemorrhages; mucosa of stomach and small intestine congested; wall of small intestine thickened; mucus and shreds of fibrin adhered to mucosa of the posterior half of the small intestine.

A dose of 0.6 cc. per kilo was given either in hard gelatine capsules or soft elastic globules to the following:

Fox No. 202.—Weight 4.3 kilos; dose in hard gelatine capsules; collapse and cessation of respiration occurred when the drug was inhaled as a result of one of the capsules dissolving in the mouth before being swallowed; artificial respiration performed and the animal recovered; manifested anorexia and constipation for two days; no worms found in feces or on autopsy examination; no conclusions as to efficacy; killed fifth day. Post-mortem: Liver enlarged; region of congestion between cortex and medulla of kidneys; mucosa of stomach and small intestines slightly congested.

Fox No. 205.—Weight 5.45 kilos; dose in hard gelatine capsules; six hookworms first day; killed fifth day; few larval ascarids present which apparently reached the intestines after treatment; 100 per cent vs. hookworms; no apparent symptoms. Post-mortem: Liver congested and tissues friable; region of congestion between cortex and medulla of kidneys; moderate congestion of mucosa of small intestine.

Fox No. 323.—Weight 3 kilos; dose in soft elastic globules; two hookworms first day; killed fifth day and no worms present; 100 per cent vs. hookworms; fed 2 hours after dosing and vomited 1 hour later. Post-mortem: Liver dark in color and several irregular white areas (apparently areas of necrosis) present on surface and in parenchyma; tissues of liver friable; tissues of kidney bulged slightly on section; mucosa of small intestine slightly congested and wall thickened.

Fox No. 326.—Weight 3.5 kilos; dose in soft elastic globules; 11 hookworms first day; killed fifth day and no worms present; 100 per cent vs. hookworms; no apparent symptoms. Post-mortem: Liver dark in color with tissues friable and blood oozing on section, tissues of kidney bulged slightly on section; pancreas congested and a few petechiae present; mucosa of duodenum greatly congested.

DISCUSSION

The anthelmintic efficiency of the various dose rates of carbon tetrachlorid used in the above experiments may be summarized as follows:

AT 0.1 CC. PER KILO.—In hard gelatine capsules given to two foxes, this dose removed 22 hookworms and left 12, an efficacy of 64.7 per cent; removed no ascarids and left 2, an efficacy of 0 per cent; removed no tapeworms and left 1, an efficacy of 0 per cent.

AT 0.2 CC. PER KILO.—In hard gelatine capsules given to five foxes, this dose removed 44 hookworms and left 1, an efficacy of 97.7 per cent; removed 24 ascarids and left 3, an efficacy of 89 per cent.

AT 0.25 CC. PER KILO.—In soft elastic globules given to three foxes, this dose removed 56 hookworms and left 5, an efficacy of 91.8 per cent; removed 28 ascarids and left 1, an efficacy of 96.5 per cent; removed 8 intestinal flukes and left none, an efficacy of 100 per cent.

AT 0.3 CC. PER KILO.—In hard gelatine capsules or soft elastic globules given to nine foxes, this dose removed 330 hookworms and left 7, an efficacy of 97.9 per cent; removed 46 ascarids and left 1, an efficacy of 97.8 per cent; removed 2 intestinal flukes and left none, an efficacy of 100 per cent. Administered in hard gelatine capsules to four foxes, this dose removed 316 hookworms and left 3, an efficacy of 99 per cent; removed 1 ascarid and left none, an efficacy of 100 per cent. Given in soft elastic globules to five foxes, this dose removed 14 hookworms and left 4, an efficacy of 77.7 per cent; removed 45 ascarids and left 1, an efficacy of 97.8 per cent; removed 2 intestinal flukes and left none, an efficacy of 100 per cent.

AT 0.35 CC. PER KILO.—In soft elastic globules given to two foxes, this dose removed 91 hookworms and left none, an efficacy of 100 per cent; removed 37 ascarids and left 1, an efficacy of 97.3 per cent.

AT 0.4 CC. PER KILO.—In soft elastic globules given to two foxes, this dose removed 13 hookworms and left none, an efficacy of 100 per cent; removed 2 ascarids and left none, an efficacy of 100 per cent.

AT 0.45 CC. PER KILO.—In soft elastic globules given to two foxes, this dose removed 195 hookworms and left 1, an efficacy of 99.4 per cent; removed 1 ascarid and left none, an efficacy of 100 per cent; removed 2 immature Physaloptera and left none, probably an efficacy of 100 per cent.

AT 0.5 CC. PER KILO.—In soft elastic globules given to two foxes, this dose removed 35 hookworms and left none, an efficacy of 100 per cent; removed 71 ascarids and left 2, an efficacy of 97.2 per cent.

AT 0.6 CC. PER KILO.—In hard gelatine capsules or soft elastic globules given to four foxes, this dose removed 19 hookworms and left none, an efficacy of 100 per cent.

In agreement with the findings of Hall (*2*) and Allen (*1*), these experiments indicate that administration of carbon tetrachlorid is attended with a relatively high degree of efficacy against hookworms and ascarids. They indicate also that this drug is effective against intestinal flukes and Physaloptera, but that it is ineffective against tapeworms.

On the whole, the drug was found about as effective in soft elastic globules as in hard gelatine capsules. In the case of foxes No. 334, 356, and 357, feed and water were given about 1¼ hours after treatment. It is very probable that the dose had not left the stomach and that the feed and water tended to dilute and mask the drug, thus reducing the efficacy of the treatment. Observations made later by the writers but reported in a previous paper (*6*) tended to indicate that feed and water probably should be withheld from foxes for about three hours after the administration of the drug in soft elastic globules, in order to allow sufficient time for the dose to escape from the globules and leave the stomach.

In two instances (foxes No. 200 and 202) no worms were found either in the feces or on post-mortem, although a microscopic examination of the feces just before treatment had shown the presence of hookworm eggs. Hall (*2*) reported having had the same experience. The chances are that both animals were infested with hookworms and that the treatment was 100 per cent efficient in both cases.

In another case (fox No. 730) two immature Physaloptera were passed and none was present on post-mortem. There is a possibility that this animal harbored more of these worms, which infest the stomach, but which were destroyed and digested to such an extent that they were unrecognizable when passed in the feces. According to Hall and Schillinger (*4*), worms infesting the stomach are liable to be destroyed by carbon tetrachlorid and digested instead of being voided in the feces.

Of the 13 foxes which were given carbon tetrachlorid in hard gelatine capsules, one collapsed from inhalation of the drug, but under artificial respiration, recovered. On the other hand, none of the 18 animals which were given the drug in soft elastic globules suffered inhalation-collapse. The writers (*6*) have found that in administering the drug cases of inhalation-collapse were not so likely to be encountered in the use of soft elastic globules as in the use of hard gelatine capsules.

In the above experiments, four of the animals died, but carbon tetrachlorid apparently was responsible for the death of only three. Since both of the 1-month-old pups died, the indications are that carbon tetrachlorid at a dose rate of 0,3 cc. per kilo is not tolerated by nursing pups. The constipation which occurred in these two animals undoubtedly resulted in increased absorption of the drug. Probably there were two other factors which predisposed them to intoxication, one being age susceptibility and the other the diet, which consisted of the milk of the vixen. The few analyses which have been made of the milk of fox vixens indicate that it is very rich in fat. Lamson et al. (*3*) found that dog puppies were more susceptible to carbon tetrachlorid than full grown

dogs and that the administration of the drug after giving digestible fatty substances such as cream increases the degree of intoxication.

Fox No. 500 manifested lesions which were more or less characteristic of those observed in foxes dying from 1 to 5 days after treatment. The writers have elsewhere (*6*) called attention to the fact that although many foxes tolerate large doses or repeated treatment, a loss of approximately 4 per cent was suffered from what may be called a delayed intoxication. The doses which these foxes received ranged from 0.3 cc. to 0.54 cc. per kilo. In the majority of these fatalities the dose varied but slightly from 0.4 cc. per kilo. It should be mentioned, however, that many foxes were dosed at the above range of rates and manifested no apparent unfavorable symptoms. Our experience has been that tolerance for carbon tetrachlorid varies not only in different foxes but in the same fox at different times.

Although pathological changes seemed to appear more constantly in the use of larger doses, the indications are that other factors than the dosage are involved in absorption of the drug. Some of these factors probably are: (*a*) Intestinal stasis, sometimes following treatment with the drug, as reported by Allen (*1*); (*b*) diseases, especially those associated with an acute enteritis; (*c*) character of the feed before and after treatment; and (*d*) idiosyncrasy of some foxes to the drug.

Even though most adult foxes and pups over 3 months old will tolerate relatively large doses of the drug, it is probably advisable to make a practice of restricting the dosage to a range of 0.2 cc. to 0.3 cc. per kilo. Reasons for this are: (*a*) This range is usually attended with a relatively high anthelmintic efficacy against the more common intestinal worms of foxes; (*b*) the danger of systemic absorption of the drug probably tends to vary according to the amount of the drug used; and (*c*) reinfestation with hookworms and ascarids, especially the former, usually occurs within a few weeks, a factor frequently requiring a repetition of treatment.

In conjunction with carbon tetrachlorid it is undoubtedly advisable to use a satisfactory purgative. The senior author has been conducting tests in which foxes were given a solution of Epsom salts by means of a stomach tube about two hours after the administration of the carbon tetrachlorid in soft elastic globules. Not giving the salts until two hours after such administration of the drug overcomes the possibility of the purgative leaving the stomach before the globules dissolve and release their contents. This method of treatment has been found effective in reducing losses from intoxication, but since it requires catching and dosing the foxes twice in connection with each treatment, a more practical method would be either to use soft elastic globules containing both carbon tetrachlorid and a satisfactory purgative or to administer carbon tetrachlorid and a saturated solution of Epsom salts at the same time by means of a stomach tube. Recent papers by Lambert (*7*) in the treatment of human patients, and by Hall and Shillinger (*5*) in the treatment of dogs, indicate that carbon tetrachlorid is best administered with a saturated solution of Epsom salts given at the same time, the drug being safer and the efficacy equally high.

CONCLUSIONS

Carbon tetrachlorid in doses of 0.2 cc. or more per kilo was found very effective in the removal of hookworms and ascarids from foxes. At a dose rate of 0.25 cc. or more per kilo, the drug was 100 per cent efficient against intestinal flukes.

The drug proved about as effective in soft elastic globules as in hard gelatine capsules; that is, when feed and water were not given until three hours after the treatment.

Although many foxes will tolerate very large doses of carbon tetrachlorid, indications are that it is not advisable to use doses in excess of 0.3 cc. per kilo.

One-month-old fox pups do not tolerate carbon tetrachlorid alone at a dose rate of 0.3 cc. per kilo.

Carbon tetrachlorid, like most anthelmintics, should be used with caution on sick animals.

The use of a satisfactory purgative, such as a saturated solution of Epsom salts, is advised in conjunction with the administration of carbon tetrachlorid to foxes.

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THE CHEMICAL COMPOSITION OF EDIBLE VISCERA FROM MEAT-PRODUCING ANIMALS[1]

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A study of the nutritive value of the edible viscera of meat-producing animals has been in progress in these laboratories for some time. Results pertaining to the antineuritic vitamin content of these organs have already been published by one of us (7),[2] while subsequent papers will probably deal with their value as sources of the other vitamins and with the biological value of their proteins. The present paper is concerned with the chemical composition of the edible viscera, upon which subject there seems to be but little information in the literature.

The data which are here contributed represent the results of fairly extensive analyses of livers and of less extensive analyses of hearts, brains, kidneys, tongues, pancreas, spleens, lungs, and stomachs from the principal types of meat-producing animals, namely, cattle, hogs, and sheep. In some cases separate analyses were made of a number of specimens of the given organ, while in other cases the analyses were made upon composite samples made up from a number of individual organs.

SOURCE AND PREPARATION OF ANALYTICAL MATERIAL

The animals supplying the experimental material were selected from among average fat steers, butcher hogs, medium weight veal calves, and fat lambs that were being slaughtered at local abattoirs.[3] The organ to be studied was removed from the slaughtered animal and chilled, and when thoroughly cold it was transported directly to the laboratory, where, after removal of extraneous fat and connective tissue, it was passed several times through a meat chopper and the ground material thoroughly mixed to form a homogenous sample for analysis. The determinations of such constituents as are subject to change through enzymatic or bacterial action were begun immediately and carried on with dispatch to a point where no further change could occur. The other determinations were begun at convenience within a day or two, the sample being stored in the interim in a closed glass container at a temperature of 34° F. Analytical work was generally begun within 24 hours after the slaughter of the animal.

METHODS OF ANALYSIS

The analytical methods employed have for the most part been amply described in the literature and had previously given satisfactory results in the hands of the authors. Appropriate references to or descriptions of these methods are given below.

Moisture.—Moisture was determined by freezing a weighed portion of the sample and drying it to constant weight in vacuo over sulphuric acid.

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[2] Reference is made by number (italic) to "Literature cited," p. 346.

[3] Valuable assistance in the selection of these animals was rendered by Dr. H. K. Walter, of the Bureau of Animal Industry, inspector in charge of meat inspection at Washington, D. C., to whom the authors acknowledge their indebtedness.

Ether extract.—The residue remaining from the moisture determination was pulverized and extracted with absolute ether in a Soxhlet apparatus. The extracted material was weighed directly in the extraction flask after the ether had been driven off and the residue had been brought to constant weight at 100° C.

Total nitrogen.—Total nitrogen was determined by the Kjeldahl-Gunning method.

Purin nitrogen.—Purin nitrogen was determined by the method of Krüger and Schittenhelm (*9*) as modified by Fellenberg (*3*).

Glycogen.—Glycogen was determined by the method of Pflüger as described by Grube (*5*).

Dextrose.—Dextrose was determined by the method of Hoagland (*6*).

Preparation of cold-water extract.—The cold-water extracts were prepared by macerating 50 grams of sample with a convenient amount of water, and washing the mixture into a volumetric flask with sufficient water to bring the final volume to 1,000 cc. The suspension thus obtained was shaken about eight times in the course of 24 hours and filtered through a dry, folded filter. The water employed in preparing the extract was cooled before used to a temperature of 2° C., and the operations described were conducted in a cold-storage room maintained at the same temperature. The filtered extract served for the determination of total creatinin and of inorganic phosphorus.

Total creatinin.—Total creatinin was determined in 200 cc. of the cold-water extract by the Folin method (*4*).

Inorganic phosphorus.—Inorganic phosphorus was determined in 200 cc. of the cold-water extract by the method of Chapin and Powick (*2*), "Modification B" of this method being employed.

Total phosphorus.—Total phosphorus was determined essentially by an adaptation of the Lorenz (*10*) method. When other ash constituents were not determined, the procedure was identical with that which has been described elsewhere (*8*) in connection with the analysis of muscular tissue. When a more or less complete ash analysis was made, an aliquot of the solution obtained from the wet combustion of a much larger sample was employed for this determination.

Preparation of solution for determination of ash constituents.—For the determination of the ash constituents, 100 grams of the prepared sample were digested with a nitric-sulphuric acid mixture in a Kjeldahl flask, care being taken to employ minimal quantities of sulphuric acid. As a rule a satisfactory combustion could be obtained by employing about 150 cc. of fuming nitric acid, added in small portions, and 10 cc. of concentrated sulphuric acid. After complete destruction of the organic matter, the clear solution was diluted to a volume of 250 cc. in a volumetric flask, and a 10 cc. aliquot was removed for the determination of total phosphorus by the method indicated. The main portion of the solution, together with washings from the pipette, was transferred to a 400 cc. beaker and evaporated, the residue being heated cautiously until the free sulphuric acid had been volatilized. The material was then dissolved in 5 cc. of concentrated hydrochloric acid and diluted with water to a volume of 100 cc. (Solution A.)

Copper.—When copper was determined, Solution A was heated to incipient boiling and saturated with hydrogen sulphid in an Erlenmeyer flask, which was then stoppered and allowed to stand over night. The copper sulphid was then filtered off, washed with hydrogen-sulphid water, and used for the determination of copper by Lowe's iodometric method (*1, p. 79*).

Separation of metals and alkaline earths from alkalies.—The filtrate from the copper sulphid was boiled to expel hydrogen sulphid and was treated with a little nitric acid to insure the oxidation of iron to the ferric condition; or when copper was not determined Solution A was treated directly with nitric acid. The solution, which always contained a large excess of phosphoric acid, was then made

weakly ammoniacal and boiled in order to precipitate the phosphates of the heavy metals and calcium. The filtrate and washings from this precipitate were then treated with a large excess of ammonia and allowed to stand over night to complete the precipitation of magnesium. The filtrate from the magnesium precipitate was reserved for the determination of the alkalies (Solution B), while the combined phosphate precipitates were dissolved in hydrochloric acid to form Solution C, which served for the determination of the heavy metals and the alkaline earths.

Determination of iron.—When iron was determined, Solution C, was rendered slightly alkaline with ammonia, and the precipitate thus formed was redissolved by the careful addition of hydrochloric acid. Iron and aluminum phosphates were then precipitated by adding the appropriate quantity of ammonium acetate to the boiling solution. The precipitate thus obtained was filtered off, dissolved in acid, and reprecipitated in the same manner. The second precipitate was filtered off, washed, and dissolved in dilute acid, after which iron was determined by reduction with metallic zinc and titration with decinormal permanganate.

Determination of alkaline earths.—Solution C, or, where iron was determined, the combined filtrates from the iron determinations, were freed from interfering metals and from P_2O_5, and used for the determination of calcium and magnesium as described in the methods adopted by the Association of Official Agricultural Chemists for the determination of manganese, calcium, and magnesium in inorganic plant constituents (*1*, *p. 17*), except that the calcium was estimated by titrating the calcium oxalate in sulphuric-acid solution with decinormal potassium permanganate.

Determination of alkali metals.—Solution B, which served for the determination of the alkali metals, was brought to a volume of 250 cc. in a volumetric flask. A 100 cc. aliquot of this solution was transferred to a 200 cc. volumetric flask and rendered neutral to methyl red, after which it was treated with 4 cc. of 5 N ammonium acetate, 2 cc. of 5 N acetic acid, and a slight but definite excess of ferric chlorid. The solution was diluted to exactly 200 cc. and filtered through a dry, folded filter from the bulky precipitate of ferric phosphate. From 100 cc. of this filtrate, the excess iron was removed as the basic acetate by boiling and filtering; after which the new filtrate, combined with the washings, was treated with a few drops of dilute sulphuric acid and brought to dryness, the residue being ignited cautiously to expel the ammonium salts. The residue was then dissolved in a small quantity of water, treated with small quantities of ammonium hydroxid and ammonium carbonate, and the solution filtered into a weighed platinum dish. After evaporation the residue of sodium and potassium sulphate was brought to constant weight and weighed. Potassium was determined by the Lindo-Gladding method (*1*, *p. 12*) and sodium by difference.

In general, all determinations were made in duplicate and the average of closely agreeing duplicate determinations is reported.

TABLE I.—*Composition of livers, expressed in terms of percentages of fresh material*

Serial No.	Description of sample	Moisture	Ether extract	Total nitrogen	Purin nitrogen	Total creatinin nitrogen	Glycogen	Dextrose	Total carbohydrate expressed as dextrose	Total phosphorus	Inorganic phosphorus	Organic phosphorus	Copper	Iron	Calcium	Magnesium	Potassium	Sodium
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
513	Ox liver	72.28	2.04	3.18	0.110	-------	0.14	2.24	2.40	0.372	0.125	0.247	0.0016	0.006	0.006	0.021	0.303	0.091
514	----do	70.40	2.10	3.22	.149	-------	.76	2.84	3.66	.372	.096	.276	.0052	.005	.005	.019	.289	.079
515	----do	68.68	2.00	3.17	.124	0.015	2.51	3.20	5.90	.345	.096	.249	.0032	-------	.006	.020	.295	.075
516	----do	67.56	4.86	3.00	.121	.012	2.92	2.57	5.72	.320	.088	.231	.0050	-------	.005	.019	.291	.077
517	----do	68.05	2.37	3.46	.110	.015	1.13	1.59	4.02	.366	.100	.266	.0057	-------	.006	.019	.274	.079
518	----do	70.79	2.53	3.30	.124	.016	.35	1.80	2.55	.376	.110	.265	.0040	-------	.006	.021	.300	.080
519	----do	68.57	4.11	3.41	.113	-------	.89	2.66	3.62	.363	.096	.267	.0049	-------	.005	.020	.292	.085
520	----do	70.88	3.83	3.26	.121	-------	.57	2.09	2.70	.336	.088	.248	.0030	-------	.005	.017	.254	.095
521	----do	73.25	2.96	3.30	.134	-------	.17	1.01	1.19	.373	.094	.279	.0027	-------	.005	.018	.235	.111
	Averages for ox livers	70.05	2.98	3.26	.123	.014	1.05	2.22	3.53	.358	.099	.259	.0039	.0056	.005	.019	.281	.086
524	Calf liver	70.05	6.15	2.92	.131	.014	.65	1.33	2.04	.351	.097	.254	.0031	-------	.0060	.020	.308	.074
525	----do	68.69	4.62	2.80	.133	.018	1.13	2.71	3.94	.349	.083	.266	.0012	-------	.0065	.020	.330	.075
526	----do	67.78	7.99	3.18	.146	.012	.59	1.19	1.83	.361	.093	.268	.0073	-------	.0065	.019	.305	.081
530	----do	71.53	4.35	3.31	-------	-------	.09	.50	.60	.355	.102	.253	.0048	-------	.0060	.020	.313	.088
531	----do	70.41	4.71	3.40	.155	-------	.36	.20	.59	.378	.109	.269	.0030	-------	.0056	.021	.308	.091
532	----do	70.50	4.63	3.31	.145	-------	.10	.32	.43	.370	.108	.262	.0076	-------	.0050	.021	.304	.086
	Averages for calf livers	69.83	5.41	3.15	.142	.015	.49	1.04	1.57	.361	.099	.262	.0045	-------	.0059	.020	.311	.082
534	Hog liver	71.88	5.45	3.32	.164	-------	0	.00	.00	.382	-------	-------	.0005	-------	.0077	.022	.306	.091
535	----do	73.21	6.41	2.92	.153	-------	0	.00	.00	.351	-------	-------	.0003	-------	.0085	.022	.289	.084
536	----do	71.76	8.11	2.91	.144	-------	0	.00	.00	.352	-------	-------	.0003	-------	.0073	.021	.315	.093
556	----do	73.71	3.55	3.23	.186	-------	0	.28	.28	.372	.098	.274	.0004	-------	.0093	.021	-------	-------
557	----do	72.23	2.90	3.48	.170	-------	0	.86	.86	.381	.098	.283	.0004	-------	.0076	.021	.284	.091
558	----do	74.25	-------	3.16	.165	-------	0	.39	.39	.375	.094	.281	.0004	-------	.0085	.020	.286	.093
	Averages for hog livers	72.84	5.28	3.17	.164	-------	0	.25	.25	.369	.097	.279	.0004	-------	.0081	.021	.296	.090

TABLE II.—*Composition of livers, expressed in terms of percentages of dry, fat-free material*

Serial No.	Description of sample	Total nitrogen	Purin nitrogen	Total creatinin nitrogen	Glycogen	Dextrose	Total carbohydrate expressed as dextrose	Total phosphorus	Inorganic phosphorus	Organic phosphorus	Copper	Iron	Calcium	Magnesium	Potassium	Sodium
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
513	Ox liver	12.38	0.428	.....	0.56	8.72	9.34	1.449	0.488	0.962	0.0061	0.025	0.024	0.081	1.180	0.356
514	....do	11.71	.542	.....	2.75	10.32	13.30	1.353	.350	1.003	.0190	.017	.020	.068	1.052	.286
515	....do	10.80	.423	0.051	8.56	10.91	20.16	1.178	.328	0.850	.0110	.....	.019	.067	1.005	.256
516	....do	10.86	.439	.043	10.59	9.32	20.77	1.160	.321	.839	.0181	.....	.020	.069	1.056	.278
517	....do	11.70	.372	.050	3.82	5.37	9.48	1.237	.338	.899	.0194	.....	.019	.066	.927	.266
518	....do	12.37	.465	.061	1.31	6.74	8.15	1.409	.413	.996	.0151	.....	.024	.078	1.122	.299
519	....do	12.48	.414	.....	3.27	9.72	13.25	1.331	.353	.978	.0180	.....	.017	.073	1.069	.310
520	....do	12.89	.478	.....	2.25	8.26	10.68	1.329	.347	.982	.0117	.....	.020	.069	1.006	.375
521	....do	13.86	.563	.....	.70	4.26	5.02	1.569	.396	1.173	.0114	.....	.019	.075	.989	.466
	Averages for ox livers	12.13	.462	.051	3.76	8.19	12.24	1.335	.370	.965	.0144	.021	.020	.072	1.045	.321
524	Calf liver	12.26	.550	.061	2.77	5.57	8.56	1.473	.407	1.066	.0129	.....	.025	.085	1.294	.309
525	....do	10.48	.498	.068	4.24	10.18	14.75	1.309	.313	.996	.0045	.....	.024	.073	1.236	.282
526	....do	13.11	.603	.049	2.44	4.91	7.55	1.490	.385	1.105	.0302	.....	.027	.077	1.259	.333
530	....do	13.71	.....	.....	.37	2.09	2.50	1.473	.423	1.050	.0201	.....	.025	.085	1.300	.365
531	....do	13.64	.622	.....	1.46	.79	2.36	1.519	.439	1.080	.0123	.....	.022	.085	1.237	.364
532	....do	13.30	.584	.....	.40	1.29	1.72	1.490	.435	1.055	.0304	.....	.020	.085	1.222	.347
	Averages for calf livers	12.77	.555	.059	1.95	4.15	6.26	1.463	.401	1.051	.0185	.....	.024	.082	1.261	.334
534	Hog liver	14.65	.725	.....	0	0	0	1.686	.....	.....	.0021	.....	.034	.097	1.353	.402
535	....do	14.33	.753	.....	0	0	0	1.722	.....	.....	.0016	.....	.042	.108	1.417	.413
536	....do	14.46	.714	.....	0	0	0	1.750	.....	.....	.0013	.....	.036	.107	1.567	.463
556	....do	14.19	.819	.....	0	1.23	1.23	1.637	.432	1.205	.0018	.....	.041	.091	.....	.....
557	....do	13.97	.683	.....	0	3.47	3.47	1.533	.395	1.138	.0016	.....	.031	.084	1.142	.365
	Averages for hog livers	14.32	.744	.....	0	.94	.94	1.665	.413	1.171	.0017	.....	.037	.097	1.369	.410

TABLE III.—*Chemical composition of various edible viscera other than liver*

Serial No.	Description of sample	Composition of undried material											Compositions of dry, fat-free material								
		Moisture	Ether extract	Total nitrogen	Purin nitrogen	Glycogen	Dextrose	Total carbohydrates expressed as dextrose	Total phosphorus	Inorganic phosphorus	Organic phosphorus	Ash	Total nitrogen	Purin nitrogen	Glycogen	Dextrose	Total carbohydrates expressed as dextrose	Total phosphorus	Inorganic phosphorus	Organic phosphorus	Ash
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
563	Ox heart	67.12	15.43	2.74	0.076	0.07	.....	.....	0.186	0.061	0.125	.....	15.70	0.44	0.43	.....	.....	1.066	0.351	0.715	.....
564	....do	64.12	20.04	2.44	.072	.08	.....	.....	.176	.053	.123	.....	15.43	.45	.53	.....	.....	1.111	.337	.774	.....
565	....do	70.24	12.00	2.61	.084	.08	.....	.....	.184	.058	.126	.....	14.69	.47	.43	.....	.....	1.036	.327	.709	.....
571	....do	75.76	4.22	2.96	.095	.04	0.28	0.32	.220	.066	.155	.....	14.77	.48	.21	1.38	1.61	1.099	.328	.771	.....
572	....do	75.92	4.21	2.87	.087	.02	.27	.30	.213	.063	.150	.....	14.45	.44	.12	1.36	1.52	1.072	.317	.755	.....
573	....do	77.15	2.74	3.01	.094	.19	.24	.45	.227	.068	.159	.....	14.98	.47	.94	1.22	2.24	1.128	.337	.791	.....
	Averages for ox hearts	71.72	9.77	2.77	.085	.08	.26	.36	.201	.061	.140	.....	15.00	.46	.44	1.32	1.79	1.085	.333	.752	.....
575	10 hog hearts	79.29	1.63	2.83	.082	.00	.19	.19	.209	.075	.134	.....	14.83	.43	.00	1.00	1.00	1.095	.391	.704	.....
576	10 lamb hearts	.....	.....	2.20	.086	.17	.23	.40	.171	.044	.127	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
588	10 ox brains	77.35	7.24	1.72	.051	.....	.00	.....	.346	.068	.278	.....	11.15	.34	.....	.00	.....	2.249	.443	1.806	.....
593	22 ox brains	77.95	6.82	1.70	.053	.....	.00	.....	.345	.051	.294	1.53	11.15	.35	.....	.00	.....	2.265	.335	1.930	10.06
	Averages for ox brains	77.65	7.03	1.71	.052	.....	.00	.....	.345	.059	.286	1.53	11.15	.34	.....	.00	.....	2.257	.389	1.868	10.06
595	50 hog brains	77.96	7.61	1.62	.039	.....	.....	.....	.359	.083	.276	1.48	11.43	.27	.....	.....	.....	2.488	.572	1.916	10.26
682	....do	78.77	7.36	1.68	.038	.....	.....	.....	.325	.070	.255	1.43	12.13	.27	.....	.....	.....	2.341	.506	1.835	10.28
	Averages for hog brains	78.36	7.48	1.65	.038	.....	.....	.....	.342	.076	.266	1.45	11.78	.27	.....	.....	.....	2.414	.539	1.875	10.27
600	70 sheep brains	78.26	7.48	1.71	.046	.....	.....	.....	.333	.071	.262	1.38	11.96	.32	.....	.....	.....	2.339	.501	1.838	9.68
604	10 beef kidneys	80.12	2.07	2.52	.083	.....	.00	.....	.230	.090	.140	1.11	14.14	.46	.....	.00	.....	1.288	.505	.783	6.21
606	60 hog kidneys	76.25	5.41	2.72	.095	.....	.....	.....	.264	.103	.161	1.18	14.81	.52	.....	.....	.....	1.439	.562	.877	6.44
612	Ox tongue	.....	.....	2.75	.072	.....	.....	.....	.158	.089	.069	0.87	.....	.....	.....	.....	.....	.....	.....	.....	.....
613	....do	.....	.....	3.03	.082	.....	.....	.....	.170	.094	.076	0.97	.....	.....	.....	.....	.....	.....	.....	.....	.....
	Averages for ox																				

613	do			3.03	.082				.170	.094	.076	0.97									
	Averages for ox tongues			2.89	.077				.164	.091	.073	0.92									
614	14 hog tongues	68.30	13.27	2.72	.081				.186	.095	.091	0.94	14.76	.44				1.008	.514	.494	5.10
618	46 lamb tongues	69.51	15.32	2.23	.058				.147	.078	.069	0.80	14.72	.39				.970	.515	.455	5.30
624	35 calf pancreas	70.44	11.10	2.63	.206				.326	.063	.263	1.25	14.26	1.12				1.766	.339	1.427	6.79
622	Hog pancreas [a]	64.12	18.40	2.54	.126				.331	.067	.264	1.19	14.55	.72				1.894	.382	1.512	6.83
631	5 ox spleens	77.48	2.26	2.94	.180				.284	.059	.225	1.41	14.52	.89				1.404	.292	1.112	6.95
629	50 hog spleens	79.00	1.88	2.76	.167				.298	.078	.220	1.44	14.46	.88				1.559	.406	1.153	7.52
638	1 pair ox lungs	78.47	2.16	2.82					.220	.054	.166	1.10	14.57					1.133	.278	.855	5.70
658	do	77.88	1.98	2.96	.109				.211	.039	.172	1.08	14.69	.54				1.047	.195	.852	5.40
	Averages for ox lungs	78.17	2.07	2.89	.109				.215	.046	.169	1.09	14.63	.54				1.090	.236	.854	5.55
635	5 pairs lamb lungs	78.00	2.36	2.90	.133				.180	.075	.105	1.30	14.76	.68				.915	.382	.533	6.59
654	Ox stomach, [b] (cooked)	81.08	2.39	2.65	.023				.040	.003	.037	.22	16.01	.14				.243	.018	.225	1.35
673	Hog stomach (cooked)	73.96	8.95	2.64	.074				.118	.020	.098	.57	15.46	.43				.694	.119	.575	3.34

[a] Sample 622 was composite sample but no record was kept of the number of organs used in its preparation.
[b] Sample 654 consisted of "fresh honeycomb tripe," washed and cooked before purchase.

TABLE IV.—*Average composition of lean beef muscle*

Items	Composition expressed in terms of percentages of undried material							Composition expressed in terms of percentages of dry, fat-free material				
	Moisture	Ether extract	Total nitrogen	Total phosphorus	Inorganic phosphorus	Organic phosphorus	Ash	Total nitrogen	Total phosphorus	Inorganic phosphorus	Organic phosphorus	Ash
Average composition of lean muscle from 8 beef rounds	74.33	2.82	3.41	0.203	0.109	0.094	1.07	14.93	0.885	0.477	0.408	4.68
Average composition of lean muscle from 7 beef rumps	73.01	4.68	3.28	.195	.102	.093	1.03	14.73	.872	.457	.415	4.62
Average composition of lean muscle from 7 beef loins	72.07	6.00	3.27	.188	.098	.090	1.00	14.90	.858	.459	.409	4.57

DISCUSSION OF RESULTS

The results obtained from the analyses of the edible viscera are reported in Tables I, II, and III. For purposes of comparison, Table IV, showing the average composition of beef muscle from different parts of the carcass, has been compiled from analyses previously reported in another connection (*3*).

In general, the data embodied in Tables I, II, and III correspond with what is known regarding the physiological functions of the several organs, and with such analytical data as are to be found in the general textbooks. Since most of the organs are the seat of intense physiological activity, and are correspondingly rich in cells, while some are known to be comparatively rich in phospholipins, high percentages of phosphorus, and more especially of organically combined phosphorus, were to have been expected. The deficiency of carbohydrates in hog liver was unexpected, however, in view of the well-known function of the liver as a storage depot for glycogen; and the reason for this deficiency is not clear.

In so far as the data bear upon the question of nutritive value, they show that the edible viscera are preeminently nitrogenous foods, and as such are most properly compared with meat as a standard. A practical equivalence between lean beef muscle, and the livers of ox, calf, and hog, in this respect, is indicated, while the other organs examined were all inferior to beef muscle in their nitrogen content. With the exception of hearts, tongues, lungs, and stomachs, on the other hand, the edible viscera examined were found to be richer in phosphorus than is beef muscle.

The chemical composition of a food, however, can no longer be regarded as constituting in and of itself a sufficient index of its nutritive value; so that further discussion of the nutritive value of the edible viscera will be postponed until the studies now in progress on the biological value of their proteins and on their value as sources of the several vitamins shall have been completed.

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FUMIGATION OF BEAN WEEVILS, BRUCHUS OBTECTUS SAY AND B. QUADRIMACULATUS FAB.[1]

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INTRODUCTION

In the course of the bean weevil investigations carried on in California during the last four years, the writer has had an opportunity to make observations on the methods of fumigation and their effectiveness when applied to bean weevils.[2]

He has carried on fumigation experiments using carbon disulphide, hydrocyanic-acid gas obtained from liquefied hydrocyanic acid, and hydrocyanic-acid gas generated by the pot method, the amounts of beans fumigated varying from only a small quantity to 300 centals, or 30,000 pounds, at a time.

Liquefied hydrocyanic-acid gas, as used in these experiments, was the commercial hydrocyanic-acid gas. This material contains not less than 96 per cent actual hydrocyanic acid, and is free from alkalies, ammonia, chlorine, hydrochloric acid, and nitric acid. For economy of space, this material is designated as Liq. HCN in the tables of results.

The material used in the pots for the generation of hydrocyanic-acid gas was commercial sodium cyanide labeled 98–99 per cent pure and commerical sulphuric acid 66° B.

The beans, California Pink and Red Mexican, and the cowpeas, Large Blackeye, Red Ripper, and Black-Spotted or Holstein, used in these experiments, are thought to be typical examples of beans and cowpeas, and it is believed that a fumigant will penetrate other varieties in approximately the time required to penetrate these. The bean weevils, *Bruchus obtectus* Say, were contained within and among the California Pink, Red Mexican, and Large Blackeyed cowpeas, while the four-spotted cowpea weevils, *B. quadrimaculatus Fab.*, were within and among the cowpeas only.

In these experiments carbon disulphide was used in quantities varying from 1.5 to 31 pounds per 1,000 cubic feet, at temperatures of from 52° to 82° F., and with exposures of from 1 to 90 hours, as well as in a vacuum of 29 inches. Liquefied hydrocyanic acid was used in amounts from 1.3 to 3.5 pounds[3] per 1,000 cubic feet at temperatures of from 58° to 92° F. for lengths of time varying from 24 to 90 hours. In generating hydrocyanic-acid gas by the pot method, from 1.8 to 3.4 pounds of sodium cyanide was used per 1,000 cubic feet at 67° to 84° F. for from 48 to 90 hours.

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[1] Received for publication April 19, 1924.

[2] The writer began the bean weevil investigation in 1919 under the direction of Dr. E. A. Back, entomologist in charge of stored-product insect investigations of the Bureau of Entomology, United States Department of Agriculture. In the fumigation work he has been assisted at various times by E. Nelson, B. Smit, and A. H. Amis, all of whom have rendered assistance in various ways.

He wishes to thank especially D. B. Mackie, of the State department of agriculture of California, Sacramento, Calif., C. L. Main, of the Puente Milling & Warehouse Co., Puente, Calif., and E. R. Hulbirt, of the Owl Fumigation Corporation, Azusa, Calif., for their valuable cooperation, and for the services they so courteously rendered.

[3] In citrus fumigation 18 cc. of liquefied hydrocyanic acid is considered to be the equivalent of 1 ounce of sodium cyanide, but 20 cc. is more nearly the equivalent at 60° F. At 60° F., 642 cc. of liquefied hydrocyanic acid weighs 1 pound; at 70° F., 650 cc. of liquefied hydrocyanic acid weighs 1 pound; at 80° F., 659 cc. of liquefied hydrocyanic acid weighs 1 pound.

The investigations have shown that fumigation of beans as it is done frequently in warehouses, outside of warehouses under canvas covers, and in loaded freight cars does not give satisfactory results. Even in fumigators specially constructed, within or adjoining the warehouses, the kill is not always complete, and the statement is commonly made that fumigation does not kill the eggs or immature stages.

In cases where carbon disulphide was used, the unsatisfactory results are not to be wondered at when one considers the statements of scientific workers along this line. In 1895 Osborn and Mally (*4*)[4] said: "For carbon disulphide to be effective there must be two or three applications about three or four weeks apart."

Pettit (*6*), in 1906, said: "Fumigation of the seed, during a warm spell, using carbon disulphide in the regular way . . . should kill all the active beetles, and a second treatment, after a period of warm weather, should complete the work, if indeed the first does not suffice."

Krall, in 1914 (*2*), questioned the possibility of killing all immature forms with one application. Metcalf (*3*), in 1916, when reporting work done in 1912 with *Bruchus chinensis* L. and *B. quadrimaculatus*, says that he fumigated them with carbon disulphide, "using 4 pounds to 1,000 cubic feet of air space in a very tight bin at 74° for 72 hours. Contrary to our expectation, however, the cowpea weevils continued to breed in undiminished numbers, although a great many adults were killed at the time the fumigation was done."

Wade (*7*), in 1919, says: "One of the first difficulties encountered in our tests was to kill all stages of the weevil in cowpeas by fumigating with carbon disulphide for the usual length of time. It was determined before proceeding far that all of the immature stages were not overcome by the fumigant, especially where several bushels were treated."

In view of the foregoing the writer underook to determine which stage of the weevils, *B. obtectus* and *B. quadrimaculatus*, was the most resistant and which stage was least resistant to fumigation with carbon disulphide or hydrocyanic-acid gas.

EFFECT OF FUMIGATION UPON THE EMERGED ADULT

Table I shows that 2 pounds of carbon disulphide per 1,000 cubic feet at a temperature of 53° to 61° F. killed all emerged adult bean weevils, *Bruchus obtectus*, with an exposure of 24 hours. Three pounds of carbon disulphide per 1,000 cubic feet at 62° to 68° F. killed all emerged adults in 21 hours. One and one-half pounds of carbon disulphide per 1,000 cubic feet at 52° to 68° F, killed all adults in 48 hours.

[4] Reference is made by number (italic) to "Literature cited," p. 356.

TABLE I.—*The effect of fumigation on adult weevils, Bruchus obtectus*

Number of weevils	Date fumigated	Material used	Dosage per 1,000 cubic feet	Exposure	Number alive	Number dead	Temperature		Humidity	
							Maximum	Minimum	Maximum	Minimum
			Lbs.	*Hrs.*			°*F.*	°*F.*		
100	Apr. 19	CS_2	2	24	0	100	61	53	70	66
100	do	do	2	24	0	100	61	53	70	66
50	do	do	2	24	0	50	61	53	70	66
100	(a)				100	0	76	49	68	41
100	Apr. 20	CS_2	3	24	0	100	58	53	70	66
100	do	do	3	24	0	100	58	53	70	66
50	do	do	3	24	0	50	58	53	70	66
100	(a)				100	0	75	49	75	45
100	Apr. 21	CS_2	1.5	48	0	100	68	60	64	59
100	(a)				100	0	85	50	70	29
100	Apr. 23	CS_2	1.5	48	0	100	60	52	68	59
100	(a)				100	0	69	41	68	19
100	May 13	CS_2	3	21	0	100	68	62	73	71
100	(a)				100	0	81	60	82	53
50	May 17	[b]Liq. HCN	1.8	24	0	50	70	62	70	45
50	do	do	1.8	24	0	50	70	62	70	45
50	(a)				50	0	85	51	92	44
50	June 8	CS_2	6	21	0	50	68	64	70	66
50	(a)				50	0	87	50	83	50
50	June 11	Liq. HCN	3.5	48	0	50	75	62	80	58
50	do	do	3.5	48	0	50	75	62	80	58
50	do	do	3.5	48	0	50	75	62	80	58
50	(a)				50	0	83	64	82	54
50	(a)				50	0	83	64	82	54
50	(a)				50	0	83	64	82	54

[a] Control.
[b] All fumigation by the use of liquified hydrocyanic acid was done in cooperation with E. R. Hulbirt of the Owl Fumigation Corporation of Azusa, Calif.

Table II shows that all emerged adult four-spotted cowpea weevils, *Bruchus quadrimaculatus*, were killed by an exposure of 24 hours to 1.8 pounds of liquefied hydrocyanic acid at 62° to 70° F., and that 1.3 pounds was equally effective when the exposure was extended to 90 hours at a temperature ranging from 58° to 91° F. In a 29-inch vacuum where carbon disulphide was used at the rate of 31 pounds per 1,000 cubic feet at a temperature of 82° F. all emerged adults were killed in one hour.

TABLE II.—*The effect of fumigation on adult weevils, Bruchus quadrimaculatus*

Number of weevils	Date fumigated	Material used	Dosage per 1,000 cubic feet	Exposure	Number alive	Number dead	Temperature		Humidity	
							Maximum	Minimum	Maximum	Minimum
			Lbs.	*Hrs.*			°*F.*	°*F.*		
50	May 17	Liq. HCN	1.8	24	0	50	70	62	70	45
50	(a)				50	0	85	51	92	44
50	Aug. 16	Liq. HCN	2	48	0	50	92	64	70	46
50	do	do	2	48	0	50	92	64	79	46
50	(a)				48	2	92	64	79	46
50	(a)				47	3	92	64	79	46
50	Aug. 17	CS_2 and 29-inch vacuum.[b]	31	1	0	50	82	82		
50	do	do	31	1	0	50	82	82		
50	(a)				50	0	92	64	79	46
50	(a)				50	0	92	64	79	46
50	Aug. 18	Liq. HCN	1.3	90	0	50	91	58	79	43
50	do	do	1.3	90	0	50	91	58	79	43
50	(a)				45	5	91	58	79	43
50	(a)				46	4	91	58	79	43
50	Aug. 23	HCN, pot method [c]	[d]3.4	48	0	50	82	69	71	51
50	do	do	[d]3.4	48	0	50	82	69	71	51
50	do	do	[d]3.4	48	0	50	82	69	71	51
50	(a)				50	0	82	69	71	51
50	Aug. 25	HCN, pot method [c]	1.8	90	0	[e]50	84	67	66	51
50	(a)				[e]50	0	84	67	66	51

[a] Control.
[b] In the experiments with vacuum fumigation the material was prepared by the writer and fumigated by Mr. D. B. Mackie, of the California State Department of Agriculture, after which tabulations were made by the writer.
[c] For economy of space in the tables, "HCN, pot method" is used to designate hydrocyanic-acid gas generated by the pot method.
[d] Dosage of sodium cyanide employed to generate the gas.
[e] These weevils emerged on Aug. 24

EFFECT OF FUMIGATION UPON THE EGG

Although Table III shows that 2 pounds of carbon disulphide per 1,000 cubic feet at 53° to 61° F. for 24 hours did not kill all the eggs of *Bruchus obtectus*, it shows that 3 pounds at 53° to 58° F. prevented the hatching of any eggs, and 1½ pounds at temperature only slightly higher killed all eggs in 48 hours. One and eight-tenths pounds of liquefied hydrocyanic acid per 1,000 cubic feet prevented the hatching of any eggs when exposed 24 hours at 62° to 70° F.

TABLE III.—*Effect of different kinds of fumigation for varying lengths of time on eggs of Bruchus obtectus*

Number of eggs	Date deposited	Date treated	Material used	Dosage per 1,000 cubic feet	Exposure	Number hatched	Number failed to hatch	Temperature		Humidity	
								Maximum	Minimum	Maximum	Minimum
				Lbs.	*Hrs.*			° *F.*	° *F.*		
100	Apr. 18	Apr. 19	CS_2	2	24	10	90	61	53	70	66
50	do	do	do	2	24	0	50	61	53	70	66
100	do	(a)				89	11	76	49	68	41
50	do	(a)				49	1	76	49	68	41
100	Apr. 19	Apr. 20	CS_2	3	24	0	100	58	53	69	66
100	do	(a)				94	6	75	49	75	45
100	Apr. 20	Apr. 21	CS_2	1.5	48	0	100	68	60	64	59
50	do	do	do	1.5	48	0	50	68	60	64	59
50	do	(a)				46	4	85	50	70	29
50	Apr. 22	Apr. 23	CS_2	1.5	48	0	50	60	52	68	59
50	do	(a)				41	9	69	41	68	19
100	May 12	May 13	CS_2	3	21	0	100	68	62	73	71
100	do	(a)				83	17	81	60	82	53
50	May 16	May 17	Liq. HCN	1.8	24	0	50	70	62	70	45
50	do	do	do	1.8	24	0	50	70	62	70	45
50	do	(a)				47	3	85	51	92	44
50	June 7	June 8	CS_2	6	21	0	50	68	64	70	66
50	do	do	do	6	21	0	50	68	64	70	66
50	do	(a)				46	4	87	50	83	50
50	June 10	June 11	Liq. HCN	3.5	48	0	50	75	62	80	58
50	do	do	do	3.5	48	0	50	75	62	80	58
50	do	do	do	3.5	48	0	50	75	62	80	58
50	do	do	do	3.5	48	0	50	75	62	80	58
50	do	(a)				44	6	83	64	82	54
50	do	(a)				46	4	83	64	82	54
50	do	(a)				44	6	83	64	82	54

[a] Control.

The most striking thing shown in Table IV is that at a temperature of 62° to 70° F. the viability of all the eggs of *Bruchus quadrimaculatus* was not destroyed by the use of 1.8 pounds of liquefied hydrocyanic acid per 1,000 cubic feet for 24 hours, although the same dosage (see Table III) killed all eggs of *Bruchus obtectus*.

The eggs recorded in Tables III and IV were so arranged that there were eggs which had been deposited less than 24 hours, other eggs in every stage of development, and some just beginning to hatch.

TABLE IV.—*Effect of different kinds of fumigation for varying lengths of time on eggs of Bruchus quadrimaculatus*

Number of eggs	Date deposited	Date treated	Material used	Dosage per 1,000 cubic feet	Exposure	Number hatched	Number failed to hatch	Temperature		Humidity	
								Maximum	Minimum	Maximum	Minimum
				Lbs.	*Hrs.*			°*F.*	°*F.*	°*F.*	°*F*
50	May 16	May 17	Liq. HCN	1.8	24	0	50	70	62	70	45
50	do	do	do	1.8	24	0	50	70	62	70	45
50	do	(a)				42	8	85	51	92	44
39	do	May 17	do	1.8	24	1	38	70	62	70	45
38	do	(a)				28	10	85	51	92	44
50	June 7	June 8	CS_2	6	21	0	50	68	64	70	66
50	do	do	do	6	21	0	50	68	64	70	66
50	do	(a)				43	7	87	50	83	50
50	do	(a)				49	1	87	50	83	50
50	June 10	June 11	Liq. HCN	3.5	48	0	50	75	62	80	58
50	do	do	do	3.5	48	0	50	75	62	80	58
50	do	do	do	3.5	48	0	50	75	62	80	58
50	do	do	do	3.5	48	0	50	75	62	80	58
50	do	(a)				47	3	83	64	82	54
50	do	(a)				49	1	83	64	82	54
50	do	(a)				43	7	83	64	82	54
50	Aug. 15	Aug. 16	Liq. HCN	2	48	0	50	92	64	79	46
50	do	do	do	2	48	0	50	92	64	79	46
50	do	(a)				44	6	92	64	79	46
50	do	(a)				36	14	92	64	79	46
50	do	Aug. 17	CS_2 and 29 inch-vacuum.	31	1	0	50	82	82		
50	do	do	do	31	1	0	50	82	82		
50	do	(a)				44	6	92	64	79	46
50	do	(a)				36	14	92	64	79	46
50	Aug. 17	Aug. 18	Liq. HCN	1.3	90	0	50	91	58	79	43
50	do	do	do	1.3	90	0	50	91	58	79	43
50	do	(a)				[b] 37	13	91	58	79	43
50	do	(a)				[b] 35	15	91	58	79	43
50	Aug. 22	Aug. 23	HCN, pot method.	[c] 3.4	48	0	50	82	69	71	51
50	do	do	do	[c] 3.4	48	0	50	82	69	71	51
50	do	(a)				[b] 37	13	82	69	71	51
50	do	(a)				[b] 35	15	82	69	71	51
50	Aug. 24	Aug. 25	HCN, pot method.	[c] 1.8	90	0	50	84	67	66	51
50	do	do	do	[c] 1.8	90	0	50	84	67	66	51
50	do	(a)				34	[d] 11	84	67	66	51
50	do	(a)				28	[d] 22	84	67	66	51

[a] Control.

[b] Figures seem to indicate that the controls were identical on August 17 and August 22, which is not the case.

[c] Dosage of sodium cyanide employed to generate the gas.

[d] Mites, *Pediculoides ventricosus* Newp., probably account for the low hatch and in one of the experiment the five eggs unaccounted for.

EFFECT OF FUMIGATION UPON FORMS WITHIN THE SEEDS

The data in Table V indicate that 2 pounds of carbon disulphide per 1,000 cubic feet at 53° to 61° F. for 24 hours was not effective in killing the unemerged adults, pupæ, full-grown larvæ, or half-grown larvæ, of *Bruchus obtectus*, but was effective in killing the young larvæ. The same dosage gave a complete kill when adults free of seeds were treated. (See Table I.) Three pounds of carbon disulphide at 53° to 58° F. for 24 hours made a complete kill, as did 1½ pounds for 48 hours at 60° to 68° F. One and eight-tenths pounds of liquefied hydrocyanic acid per 1,000 cubic feet at 62° to 70° F. for 24 hours did not kill all the unemerged adults and pupæ, but it killed the larvæ. The same dosage killed all eggs of *B. obtectus* and emerged adults of both species of weevils. (See Tables I to IV.)

TABLE V.—*Percentage of weevils, Bruchus obtectus, that were dead within the beans after being fumigated with different amounts of carbon disulphide or hydrocyanic-acid gas for varying lengths of time* [a]

Amount fumigated	Material used	Dosage per 1,000 cubic feet	Exposure	Unemerged adults		Pupæ		Larvæ						Temperature		Humidity	
								Full-grown		Half-grown		Young					
				Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Maximum	Minimum	Maximum	Minimum
Centals.		*Lbs.*	*Hrs.*											°*F.*	°*F.*		
13	CS_2	2	24	58	42	54	46	6	94	6	94	0	100	61	53	70	66
	Control			84	16	81	19	60	40	50	50	0	0	76	49	68	41
13	CS_2	2	24	37	63	38	62	25	75	0	0	0	0	61	53	70	63
	Control			82	18	98	2	67	33	0	0	0	0	76	49	68	41
9	CS_2	3	24	0	100	0	100	0	100	0	0	0	0	58	53	69	66
	Control			75	25	94	6	53	47	0	0	0	0	75	49	75	45
9	CS_2	3	24	0	100	0	100	0	100	0	0	0	0	58	53	69	66
	Control			64	36	88	12	100	0	0	0	0	0	75	49	75	45
9	CS_2	1.5	48	0	100	0	100	0	100	0	0	0	0	68	60	64	59
9	do	1.5	48	0	100	0	100	0	100	0	0	0	0	68	60	64	59
	Control			70	30	61	39	79	21	0	0	0	0	85	50	70	29
9	CS_2	1.5	48	0	100	0	100	0	100	0	0	0	0	60	52	68	59
	Control			85	15	90	10	0	0	0	0	0	0	69	41	68	19
9	CS_2	1.5	48	18	82	13	87	11	89	0	0	0	0	60	52	68	59
	Control			82	18	92	8	100	0	0	0	0	0	69	41	68	19
5	CS_2	3	21	5	95	9	91	35	65	0	0	0	0	68	62	73	71
	Control			55	[b] 45	59	[b] 41	5	[b] 95	0	0	0	0	81	60	82	53
210	CS_2	6	21	[c] 2	98	26	74	40	60	0	0	0	0	68	64	70	66
	Control			97	3	86	14	71	29	0	0	0	0	87	50	83	50
210	CS_2	6	21	[c] 20	80	47	53	0	0	0	0	0	0	68	64	70	66
	Control			98	2	87	13	67	33	0	0	0	0	87	50	83	50
210	CS_2	6	21	[c] 5	95	35	65	10	90	0	0	0	0	68	64	70	66
210	do	6	21	[c] 6	94	60	40	71	29	0	0	0	0	68	64	70	66
	Control			90	10	97	3	62	38	0	0	0	0	87	50	83	50
	do			96	4	90	10	75	25	0	0	0	0	87	50	83	50
300	Liq. HCN	1.8	24	49	51	68	32	0	100	0	0	0	0	70	62	70	45
300	do	1.8	24	38	62	56	44	0	0	0	0	0	0	70	62	70	45
300	do	1.8	24	52	48	20	80	0	100	0	0	0	0	70	62	70	45
	Control			92	8	84	16	88	12	0	0	0	0	85	51	92	44
150	Liq. HCN	3.5	48	0	100	0	100	0	100	0	0	0	0	75	62	80	58
	Control			94	6	94	6	83	17	0	0	0	0	83	64	82	54
150	Liq. HCN	3.5	48	0	100	0	100	0	100	0	0	0	0	75	62	80	50
	Control			90	10	82	18	75	25	0	0	0	0	83	64	82	54
150	Liq. HCN	3.5	48	0	100	0	100	0	100	0	0	0	0	75	62	80	50
	Control			85	15	83	17	50	50	0	0	0	0	83	64	82	54

[a] One hundred weevily beans were placed in each of a number of cotton-stoppered vials and the vials placed in bags of beans in different parts of the fumigator. A like number of weevily beans were removed to the laboratory for controls.

[b] Became heavily infested with mites, *Pediculoides ventricosus*, before they could be dissected.

[c] Six pounds of CS_2 was placed in three shallow pans. In 1.2 hours it was necessary to remove 31 bags; then 1¼ pounds of CS_2 was added, and the fumigator was closed after being open less than 5 minutes. Results should be discarded.

Table VI shows that all stages of *Bruchus quadrimaculatus* contained within the black-spotted cowpeas were killed by fumigation with 31 pounds of carbon disulphide per 1,000 cubic feet in a 29-inch vacuum for 1 hour at 82° F., with 2 pounds of liquefied hydrocyanic acid per 1,000 cubic feet for 48 hours at 64° to 92° F., and with 1.3 pounds of liquefied hydrocyanic acid at 58° to 91° F. for 90 hours. The use of 3.4 pounds of sodium cyanide in fumigation with hydrocyanic-acid gas by the pot method at 69° to 82° F. was also 100 per cent effective

TABLE VI.—*Effect of fumigation of weevils, Bruchus quadrimaculatus, within black-spotted cowpeas*

Material used	Dosage per 1,000 cubic feet	Exposure	Killed	Eggs	Larvæ				Pupæ	Adults		Temperature		Humidity	
					Young	One-fourth grown	One-half grown	Full-grown		Unemerged	Emerged before fumigation	Maximum	Minimum	Maximum	Minimum
	Lbs.	*Hrs.*	*P. ct.*									*° F.*	*° F.*		
CS_2 and 29-inch vacuum	31	1	100	172	51	12	6	15	25	24	8	82	82	----	----
Do	31	1	100	154	23	8	10	13	36	21	11	82	82	----	----
Liq. HCN	2	48	100	157	34	20	6	20	27	14	7	92	64	79	46
Do	2	48	100	210	28	17	5	26	25	17	9	92	64	79	46
Control	----	----	----	----	28	23	18	17	13	27	51	92	64	79	46
Do	----	----	----	----	171	64	15	18	28	26	47	92	64	79	46
Liq. HCN	1.3	90	100	69	39	9	7	17	24	6	4	91	58	79	43
Control	----	----	----	----	113	78	48	43	23	21	70	91	58	79	43
HCN, pot method	[a] 3.4	48	100	----	25	21	18	17	31	18	----	82	69	71	51

[a] Dosage of sodium cyanide employed to generate the gas.

The weevils here listed were in all stages from fresh-laid eggs to weevils just beginning to emerge at the time they were prepared for fumigation. The vacuum fumigation was done at Sacramento and the other at Puente, Calif. The fumigated cowpeas and controls were prepared the same day, but those going to Sacramento were in transit one day longer than the others and consequently more weevils emerged from them before fumigation. The eggs on the fumigated cowpeas were counted at the time they were dissected, but the eggs on the control were not counted because several weevils had emerged from them, thereby removing some of the eggs, and these weevils had also laid numerous eggs on the cowpeas before they were dissected.

On August 28, or 13 days after the first fumigation, the weevils within the controls were all killed, because they became infested with mites, *Pediculoides ventricosus*. Although all the weevils were dead at the time they were dissected from the cowpeas, the tabulation shows that a marked development of the weevils took place within the controls during the 13 days after the others had been killed by fumigation.

In Tables VII and VIII the weevils contained in the first four and the first ten controls, respectively, were killed because of mites when they had developed 13 days after the others were fumigated, while in the other controls the weevils were killed after 16 days' development. In these, as in Table VI, all weevils were dead when they were dissected from the cowpeas. The marked development noted in the controls indicates that all stages had been killed immediately in the fumigated cowpeas.

The work of Paddock and Reinhard (*5*) indicates that they killed all eggs, larvæ, and pupæ of *Bruchus quadrimaculatus* when they used 4 pounds of carbon disulphide per 1,000 cubic feet for only 24 hours.

TABLE VII.—*Effect of fumigation of weevils, Bruchus quadrimaculatus, within blackeyed cowpeas*

Material used	Dosage per 1,000 cubic feet	Exposure	Killed	Eggs	Larvæ				Pupæ	Adults		Temperature		Humidity	
					Young	One-fourth grown	One-half grown	Full-grown		Unemerged	Emerged before fumigation	Maximum	Minimum	Maximum	Minimum
	Lbs.	*Hrs.*	*P. ct.*									°*F.*	°*F.*		
CS_2 and 29-inch vacuum	31	1	100	237	25	3	3	5	46	31	16	82	82	----	----
Do	31	1	100	580	20	10	2	7	30	74	32	82	82	----	----
Liq. HCN	2	48	100	104	23	2	4	9	54	39	17	92	64	79	46
Do	2	48	100	76	17	5	1	3	50	42	17	92	64	79	46
Control	----	----	----	81	14	2	2	3	9	8	113	92	64	79	46
Do	----	----	----	171	22	21	22	20	21	43	105	92	64	79	46
Liq. HCN	1.3	90	100	69	20	3	0	3	44	62	7	91	58	79	43
Do	1.3	90	100	40	15	5	2	12	35	57	2	91	58	79	43
Control	----	----	----	65	9	2	1	2	18	21	110	91	58	79	43
Do	----	----	----	64	7	10	7	12	8	8	99	91	58	79	43
HCN, pot method	•3.4	48	100	----	124	53	51	91	108	128	----	82	69	71	51
Control	----	----	----	----	----	13	12	15	14	24	2,269	82	69	71	51
HCN, pot method	•1.8	90	100	78	46	5	1	3	21	51	7	84	67	66	51
Do	•1.8	90	100	68	25	15	10	1	10	49	14	84	67	66	51

• Dosage of sodium cyanide used to generate the gas.

TABLE VIII.—*Effect of fumigation of weevils, Bruchus Quadrimaculatus, within Red Ripper cowpeas*

Material used	Dosage per 1,000 cubic feet	Exposure	Killed	Eggs	Larvae				Pupae	Adults		Temperature		Humidity	
					Young	One-fourth grown	One-half grown	Full-grown		Unemerged	Emerged before fumigation	Maximum	Minimum	Maximum	Minimum
	Lbs.	*Hrs.*	*P. ct.*									°*F.*	°*F.*		
CS_2 and 29-inch vacuum	31	1	100	70	108	57	54	144	31	----	----	82	82	----	----
Do	31	1	100	63	103	54	53	138	60	----	----	82	82	----	----
Do	31	1	100	72	255	171	88	38	----	----	----	82	82	----	----
Do	31	1	100	72	163	251	75	33	1	----	----	82	82	----	----
Liq. HCN	2	48	100	169	158	72	52	60	1	----	----	92	64	79	46
Do	2	48	100	146	160	57	44	65	1	----	----	92	64	79	46
Do	2	48	100	71	508	92	18	6	----	----	----	92	64	79	46
Do	2	48	100	153	558	25	9	2	----	----	----	92	64	79	46
Control	----	----	----	----	23	73	18	18	30	66	191	92	64	79	46
Do	----	----	----	----	5	12	13	25	66	144	55	92	64	79	46
Do	----	----	----	----	37	10	5	15	25	121	160	92	64	79	46
Do	----	----	----	----	3	6	6	16	45	93	251	92	64	79	46
Liq. HCN	1.3	90	100	55	136	56	66	108	23	----	----	91	58	79	43
Do	1.3	90	100	35	100	50	71	90	29	----	----	91	58	79	43
Do	1.3	90	100	75	218	222	72	42	1	----	----	91	58	79	43
Do	1.3	90	100	54	163	257	80	53	----	----	----	91	58	79	43
Do	1.3	90	100	50	100	37	40	134	46	----	----	91	58	79	43
Do	1.3	90	100	74	256	127	78	56	8	----	----	91	58	79	43
Control	----	----	----	----	22	24	12	18	29	48	179	91	58	79	43
Do	----	----	----	----	12	14	6	12	26	53	168	91	58	79	43
Do	----	----	----	----	5		8	25	24	121	163	91	58	79	43
Do	----	----	----	----	3	28	6	10	34	114	196	91	58	79	43
Do	----	----	----	----	10	32	30	30	56	110	145	91	58	79	43
Do	----	----	----	----	6	2	3	14	25	70	203	91	58	79	43
HCN, pot method	•3.4	48	100	85	146	79	93	68	40	14	3	82	69	71	51
Do	•3.4	48	100	57	124	90	63	69	89	9	----	82	69	71	51
Do	•3.4	48	100	64	237	109	138	113	11	----	----	82	69	71	51
Do	•3.4	48	100	89	152	147	115	141	130	----	----	82	69	71	51
Control	----	----	----	----	42	41	43	47	43	72	46	82	69	71	51
Do	----	----	----	62	88	78	81	125	49	71	69	82	69	71	51
Do	----	----	----	----	19	17	41	29	39	84	100	82	69	71	51
Do	----	----	----	----	13	29	24	37	45	70	160	82	69	71	51
HCN, pot method	•1.8	90	100	79	137	92	53	159	133	1	----	84	67	66	51
Do	•1.8	90	100	70	91	62	30	106	142	19	----	84	67	66	51
Control	----	----	----	----	20	34	29	27	117	89	102	84	67	66	51
Do	----	----	----	----	12	28	16	40	60	60	131	84	67	66	51

• Dosage of sodium cyanide employed to generate the gas.

The writer's observations, together with the foregoing experiments, seem to indicate that in the commercial fumigation of beans there are four outstanding causes for the unsatisfactory results obtained. The first is the use of fumigation rooms which are far from being gas-tight, another is the use of a poor grade of carbon disulphide, and the third is carrying on the fumigation at too low temperatures. Where none of these can be given as a cause for unsatisfactory results, a fourth may enter. This is too short an exposure. Reference to the foregoing tables will indicate that a small amount of hydrocyanic-acid gas or of carbon disulphide will do the work as efficiently in from 48 to 90 hours as twice the amount of the same fumigant will in 24 hours.

With reference to carbon disulphide as a fumigant, Hinds (*1*) says:

"As a matter of fact, most, if not all, of the killing will have occurred during the first 6 hours of exposure, and the building may be ventilated after that time, as a minimum, has elapsed, though it is better to wait 12 hours or longer."

Undoubtedly this is the case when large amounts of carbon disulphide and only small quantities of beans are used, but the writer is convinced that where large quantities of beans are to be fumigated, or where relatively small amounts of the fumigant are used, a period much longer than six hours is required to make a complete kill. The writer finds that if the gas is not strong at the end of a 24-hour exposure, the room is so open that all weevils will not be killed if a large quantity of beans has been fumigated. In such a room a large quantity of the fumigant should be used, and only comparatively small quantities of beans should be fumigated.

Where hydrocyanic-acid gas is used, the room must be sufficiently tight to allow the gas to penetrate the beans before it diffuses out of the room. As this gas is not heavier than air, it requires a much longer time than does carbon disulphide to penetrate the beans thoroughly; therefore, a minimum of 48 hours should be allowed when using it.

A very satisfactory bean fumigator can be constructed at a moderate price, the cost being determined by the size and location of the fumigator. It should have double walls, floor, and ceiling, made of tongue-and-groove, 1-inch lumber lined with tarred paper, having all joints sealed. All openings should be closed with specially constructed doors of the icehouse type or other type which can be sealed air-tight.

A more expensive fumigator can be constructed of concrete or brick, having all joints properly sealed.

CONCLUSIONS

In commercial work there appears to be an unnecessary fear against the use of hydrocyanic-acid gas, because of its poisonous nature, but with the care that should be exercised in the use of carbon disulphide, hydrocyanic-acid gas can be used with safety.

In commercial work, as well as in scientific work, opinions differ as to the efficiency of carbon disulphide as a fumigant against bean weevils. While this appears to be due principally to three factors, in some instances a fourth factor may enter. These factors are:

1. The use of a room or fumigatorium which is far from being gas-tight.
2. The use of an inferior grade of carbon disulphide.
3. Carrying on the work of fumigation at too low temperatures.
4. (Which may enter when large quantities of beans are being fumigated and when unsatisfactory results can be charged to neither of the first three factors.) Too short an exposure. No. 1, above, frequently resolves itself into too short an exposure.

When using a good grade of either carbon disulphide or hydrocyanic-acid gas in a gas-tight fumigator, at a sufficiently high temperature, and with a sufficiently long exposure, it is entirely possible with one fumigation to kill all the weevils, in all stages, either among or within beans or cowpeas. This, however, will of course not prevent a reinfestation if weevils have access to the fumigated seeds.

Small quantities of beans may be thoroughly fumigated with a minimum of 3 pounds of carbon disulphide per 1,000 cubic feet at a temperature of 58° F. or higher for a period of 24 hours or with 1½ pounds for 48 hours; with two pounds of liquefied hydrocyanic acid for 48 hours at 70° F. or higher or with 1.3 pounds for 90 hours; with hydrocyanic-acid gas generated from 3.4 pounds of sodium cyanide by the pot method at 70° F. for 48 hours or from 1.8 pounds of sodium cyanide for 90 hours.

Large quantities of beans require a longer exposure or a greater quantity of the fumigant, the latter being preferable. When beans are fumigated with hydrocyanic-acid gas they should be given an exposure of at least 48 hours.

When either carbon disulphid or hydrocyanic-acid gas is used as a fumigant, the weevil *Bruchus obtectus* is most easily killed in the young larval stage just after hatching and just before entrance into the bean. Emerged adults, eggs, and young larvæ just after having entered the bean succumb to fumigation in the order named, while it appears that unemerged adults and pupæ are hardest to kill. It appears, however, that the depth to which the weevil is buried in the bean is important as affecting its ability to withstand fumigation.

Stages of *Bruchus quadrimaculatus* are most easily killed in the following order: Emerged adults, eggs, larvæ that have not entirely left the chorion, and young larvæ just inside the seed; the other stages appear to be effected almost equally. The larvæ that are deepest in the cowpeas survive longer than other larvæ. This accounts for the fact that the prepupæ appear to be a little more resistant than the other stages.

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LEATHER ROT OF STRAWBERRIES[1]

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One of the most destructive diseases of strawberries (Fragaria sp.) in the southern Mississippi Valley is a rot of the fruit caused by a fungus which seems to be identical with *Phytophthora cactorum* (Leb. et Cohn) Schroeter. Yet until the spring of 1922 this rot was apparently unknown to plant pathologists, a fact which may perhaps be taken as emphasizing the relatively slight amount of attention which has been given to diseases of this important fruit. Even to strawberry growers the disease was known merely as "water-soak," since they had observed that it was always most serious during wet weather. On the basis of facts to be presented later in the paper, it is proposed that this disease be known by the common name of "leather rot."

The writer first learned of the disease during the course of a trip through the strawberry producing sections of north-central Arkansas and western Tennessee in late April and early May, 1922. The rainfall during that time was unusually heavy and had been so for some time previous. The rot was found in every field inspected, the loss ranging from 10 per cent in some fields to 25 and even 50 per cent in others. Losses fully as heavy were noted the following year (1923) in the district around Beebe, Arkansas, and a few fields were seen in which for several days the loss amounted to three-fourths of the berries then ripening on the plants. The average loss for the whole season (1923) in this district was certainly not less than 20 per cent of the crop. In sections where it occurs, Phytophthora rot is one of the most serious diseases with which the strawberry grower has to contend and therefore deserves thorough investigation.

EARLY FIELD OBSERVATIONS AND DESCRIPTION OF THE DISEASE

In all fields examined berries were found which were somewhat softened but tough and leathery and slightly browned—all of these being symptoms well known to the growers as typical of "water soak." After further investigation it seemed certain that they were caused by the attack of a parasite. Some fruits were seen which showed a white mold on the surface while still attached to the plant; others developed the mold after being held over night in a moist chamber. Mounts from the superficial growth showed almost always large numbers of conidia that were strongly suggestive of Phytophthora and cultures from affected fruits, whether moldy or not, yielded a white fungus; the fungus was later shown to be similar to and probably identical with *Phytophthora cactorum*, and its pathogenicity proved by inoculations.

In the field, Phytophthora rot (leather rot) affects strawberry fruits at all stages, from blossoming to full maturity. On all of them it is characterized by a definite though rather slight softening of affected tissues, by both external and internal discoloration and usually by a marked bitter taste; the tissues, however, become tough and leathery, so that the softening never even approximates the mushy, leaky condition produced by Rhizopus sp. nor the "soft rot" condition produced by *Pezizella lythri* (*11*).[2] The discoloration, particularly on the outside, varies so greatly that it must be described in detail.

[1] Received for publication Feb. 9, 1924.

[2] Reference is made by number (italic) to "Literature cited," p. 374-375.

In fruits that have barely begun to enlarge after petal fall, all parts, receptacle, stamens, calyx, and even the peduncle, become dark brown over affected areas; the brown, at the edges, shades off into the natural green of the unaffected portion. Fruits mature enough to have turned red show over affected areas the same yellow to light brown at the center of the spot but with a transition from this through darker brown to purple to the natural red of the berry; ripe, fully colored fruits show sometimes no color change at all except a slight darkening of the red over affected spots, or sometimes a faint tinge of the purple which forms the transition to red on less mature fruits.

On the market only the most advanced stages are seen. Here, however, the superficial growth of mold, rare in the field, is frequently seen and will serve as a diagnostic characteristic if taken in connection with the external color changes and the internal characteristics now to be described.

In cross and longi-section (Pl. 1) strawberries attacked by Phytophthora show a marked browning of the vascular system accompanied usually by a less intense browning of all the other tissues. In very early stages vascular browning is often the only visible symptom. So far as can be told by the eye or by touch, affected tissues are not disintegrated, though as mentioned earlier they are always softened and decidedly tough and leathery. At no time is there any clear line of demarcation between diseased and healthy flesh nor can a separation of the two be made by mechanical means; it is not possible to lift or scoop out the lesion as can so easily be done with the lesions of Pezizella.

HISTORICAL REVIEW

This disease has been mentioned by the writer and his associates (*13*) in a preliminary report on field work. So far as can be learned that note and the present paper constitute the first record of Phytophthora as a parasite of strawberries in the United States. Osterwalder (*6*) found a Phytophthora on strawberries in Switzerland in 1912 which he identified as *P. omnivora*, apparently overlooking the fact that Schroeter (*10 p. 235*) in 1889 had adopted, instead of *omnivora*, the original specific name of *cactorum*, on the basis of the work of Lebert and Cohn (*4*) in 1870. The term *omnivora* had been used by de Bary in 1881 (see Wilson (*16 p. 75*) to include all members of the genus Phytophthora which were not referable to *infestans* and became in time a "waste basket" into which was thrown any unidentified Phytophthora (*16*). Wilson also makes the further statement in commenting on this record by Osterwalder and on other records by Marchal (*5*), Bubak (*2*) and Osterwalder (*7*) of the occurence of *P. omnivora* on other hosts, that "The figures and descriptions indicate that more than one species of Phytophthora may be concerned and that in all probability none of these outbreaks were really due to the species which is credited with the damage." (See also Lafferty and Pethybridge (*3*), footnote, p. 35.)

CAUSAL ORGANISM: APPEARANCE IN CULTURE

Pure cultures of the fungus were easily obtained from berries showing typical symptoms, by the following method: The berries were dipped twice in alcohol, the alcohol being burned off after each dipping; they were then cut across at the calyx end and the two halves were pulled apart with a flamed scalpel; portions of the infected "core" were then dug out from the torn surface with a freshly flamed scalpel and dropped into tubes of potato dextrose agar. More than 100 such plantings have been made, all but a very few of which gave the white, loosely matted growth characteristic of Phytophthora on this medium. The exceptions were either Botrytis or Pezizella. The fungus did not fill the tube though it occasionally grew up on the glass a centimeter or two above the surface of the agar slant. On oatmeal agar it usually made a scant, appressed growth, sometimes so scant that its presence could be told only by examination

with a hand lens. Old cultures sometimes showed a faint cream color but never turned brown and there was never any darkening of the medium. Cultures contaminated with bacteria were easily purified by inoculating them into apples and after six or eight days making a reisolation from the advancing edge of the lesion.

MICROSCOPICAL APPEARANCE

Mycelium.—The mycelium is coenocytic except when old. Hyphæ measure 4 to 7 μ in diameter, the average being about 6 μ. Young hyphæ are usually

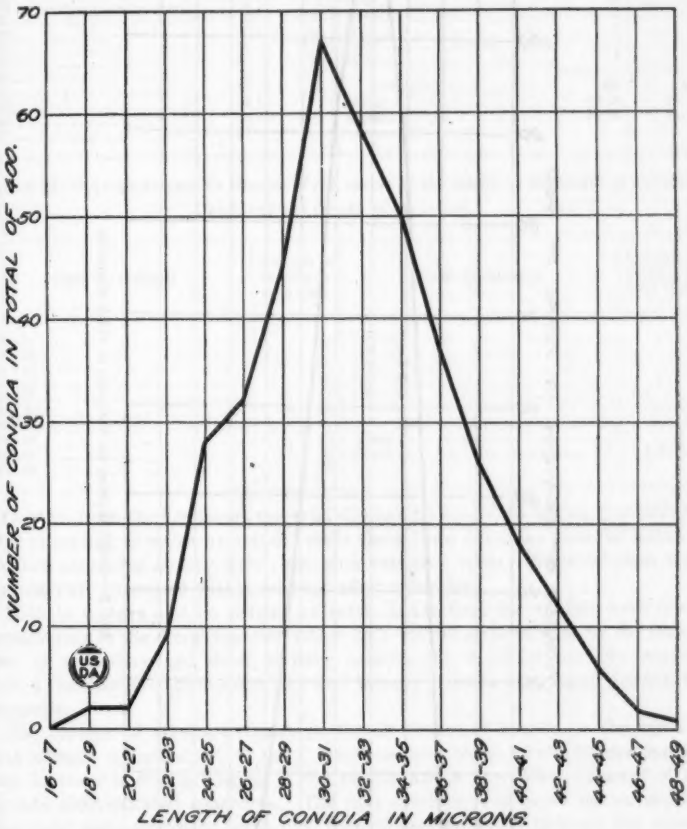

FIG. 1.—Graph showing the variation in length of conidia

full of protoplasm containing many oil drops, while old hyphæ are almost always practically empty.

Asexual stage.—In culture, conidia (Pl. 2, A, B) were produced freely at 10 to 20° C. but only in small numbers if at all, at higher temperatures. In the field they were found in abundance on fruits collected at Amite and Tickfaw, Louisiana, and at Beebe, Arkansas. Measurements of length and width of 400 conidia are given in Table I and are shown graphically in figures 1 and 2. The ratio of length to width of these conidia are given in Table II, and shown graphically in figure 3.

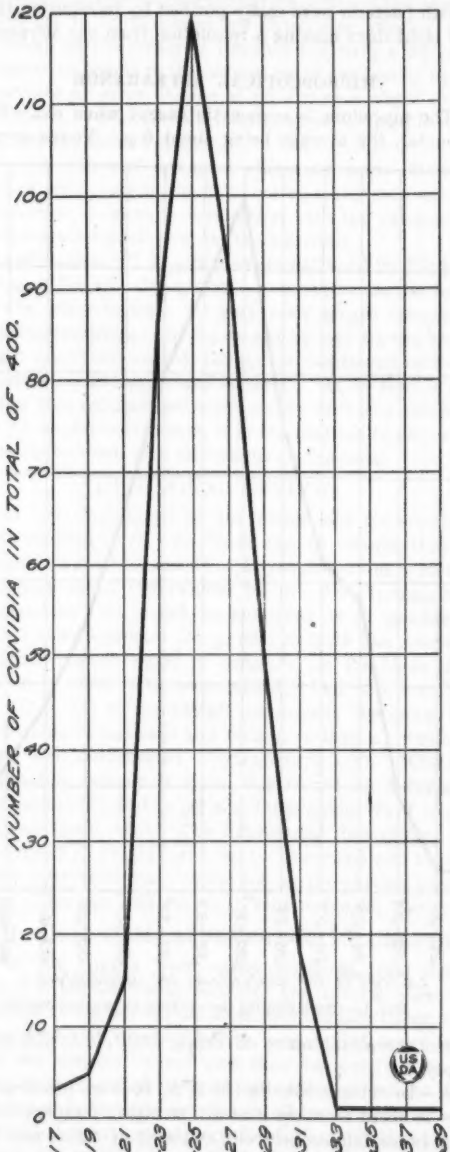

FIG. 2.—Graph showing the variation in width of conidia

TABLE I.—*Summary of measurements of conidia*

Class (in microns)	Number of conidia in each class according to—		Class (in microns)	Number of conidia in each class according to—	
	Length	Width		Length	Width
16-17		3	38-39	26	1
18-19	2	5	40-41	18	
20-21	2	23	42-43	1	
22-23	9	86	44-45	6	
24-25	28	119	46-47	2	
26-27	32	93	48-49	1	
28-29	45	49			
30-31	67	18	Total	400	400
32-33	64	1	Mean	32.26	25.09
34-35	50	1	Mode	30.31	24.25
36-37	37	1			

TABLE II.—*Arrangement in classes of the ratios of the length to the width of conidia, showing the limits of variation*

Class (in microns)	Number of conidia in each class	Class (in microns)	Number of conidia in each class
1.00-1.04	12	1.50-1.54	16
1.05-1.09	22	1.55-1.59	6
1.10-1.14	34	1.60-1.64	5
1.15-1.19	56	1.65-1.69	4
1.20-1.24	63	1.70-1.74	2
1.25-1.29	52		
1.30-1.34	44	Total	400
1.35-1.39	25	Mean	1.24
1.40-1.44	36	Mode	1.20-1.24
1.45-1.49	23		

Conidia from the Louisiana material discharged zoospores within five minutes after mounting in water on a slide, while those from Arkansas material failed to produce zoospores at any time even after repeated trials. Material from both sources were examined within an hour after collection.

Both in culture and on berries collected in the field the conidia were found germinating in the direct manner, either by a simple germ tube or by the formation of secondary and even tertiary conidia (Pl. 2, C to G). In mounts from a few cultures these secondary and tertiary conidia were found discharging zoospores.

The manner of zoospore discharge, for all classes of conidia, is the same as that already described (*8*, *9*) for *P. cactorum* and other Phytophthora species; that is, there is first a bulging of the papilla and a movement outward of the already differentiated zoospores. The first three or four move out so quickly that they seem almost to burst out, though they go singly through the narrow passage or neck at the tip of the conidium. All of them assume an hour-glass shape as they pass through the neck. The first few that come out are held together for a few seconds by the vesicle formed by the bulging out of the papilla but finally separate—presumably when the vesicle breaks, though the actual breaking was not seen—remain quiescent for a few seconds more and then swim rapidly away. The zoospores that follow the first lot come out more slowly, although the total time for what might be called normal discharge is about thirty seconds. In fact, it is almost certain that if the zoospores do not all come out within that time some of them will not come out at all. Those that remain within the conidium frequently germinate there, the germ tubes growing out through the conidium wall.

From a study of numerous mounts of conidia ripe for germination it was found that the average time for discharge to begin is about five minutes after the mount is made. After discharge the zoospores are usually active for about half an hour but finally come to rest and within 15 to 30 minutes later have germinated, the total time from discharge to germination being roughly one hour. In mounts made with a cover glass it was noted that the zoospores, whether germinating or still active, were most numerous around the edges of the cover glass, presumably

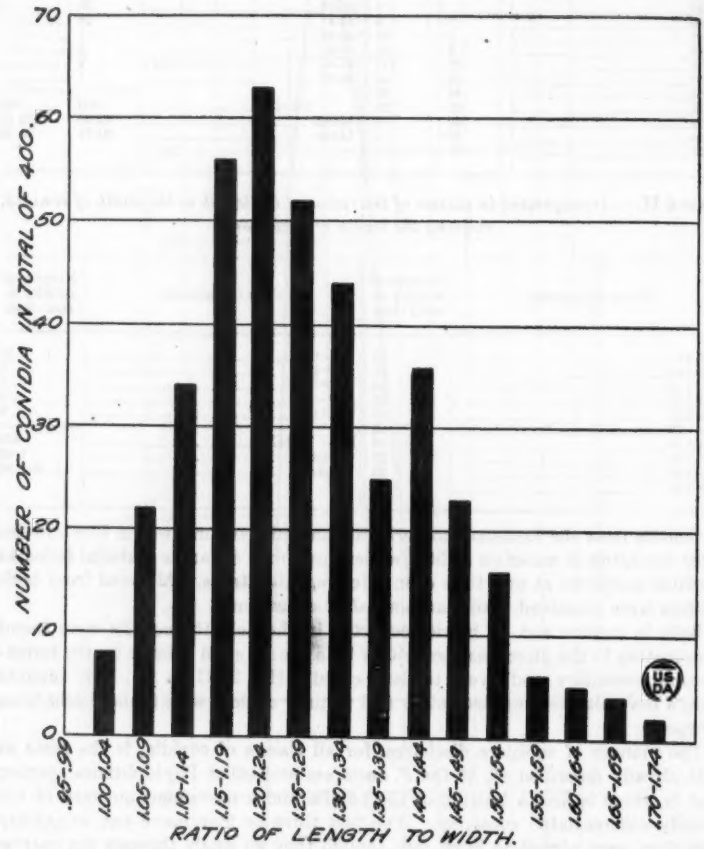

FIG. 3.—Arrangement in classes, of the ratios of the length to the width of conidia, showing the limits of variation

because of the greater supply of oxygen there. It was noted also that zoospores from conidia produced on berries in the field were much more active than those from conidia produced in cultures. The number of zoospores produced by a conidium varies from 4 to 30, the average being 15 to 18. While still active the zoospores are kidney-shaped and have two cilia; soon after they come to rest they become circular in outline and the cilia can no longer be detected. For figures illustrating the zoospores, their discharge and germination, the reader is referred to Beach (*1*, *fig. 25*) and Rosenbaum (*8*, *fig. 18*).

SEXUAL STAGE.—The sexual stage was produced in abundance on potato dextrose agar, oatmeal agar, and strawberry extract agar[3] at all temperatures from 8 to 25° C. and at the higher temperatures frequently within five days after the culture was made. It was also found on naturally infested fruits collected in the field.

Observations to date indicate that the antheridia are practically always paragynous. (Pl. 2, H to M). In old cultures on oatmeal paste a few were seen which were plainly amphigynous. The sexual organs frequently arise from the same hypha (Pl. 2, H, I, K), though they sometimes seem to arise from different hyphae (Pl. 2, J, L, M). It is possible, however, that such hyphae are merely branches having a common origin, which can not be found because the connection was broken or the hypae became badly entangled when the mount was made. The question is one which deserves further investigation.

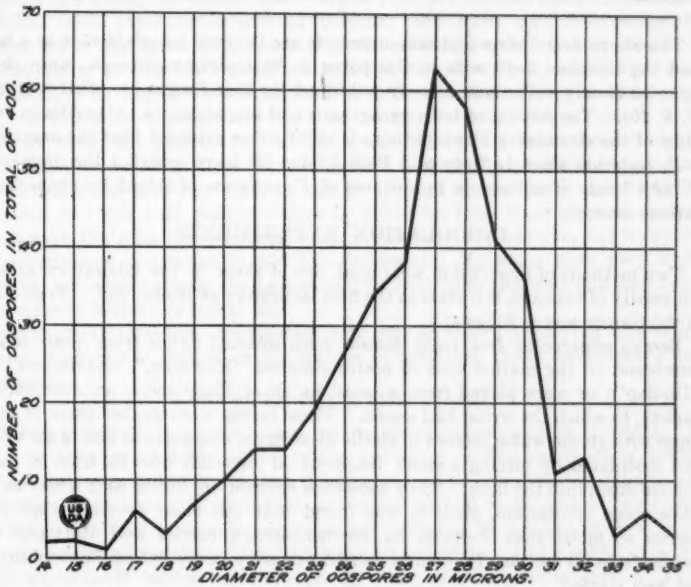

FIG. 4.—Graph showing the variation in diameter of oospores

Oogonia are hyaline, mature oospores faint yellow to sometimes a delicate orange. Germinating oospores, one of which is shown in Plate 2, N, have been observed a few times in old cultures on oatmeal agar.

Measurements of 400 oospores made on material from oatmeal agar cultures held at room temperatures for 20 days, are given in Table III, and shown graphically in figure 4.

SPHAERO-CONIDIA.—In cultures 4 to 6 months old on potato dextrose agar a few bodies have been seen which resemble the sphaero-conidia described and figured by Lafferty and Pethybridge (*3, p. 37*, and Pl. 2, fig. 13) for *P. cactorum* and *P. fagi*. These bodies vary in diameter from 26 to 44 μ and average about 33 μ. They have not been seen on diseased berries.

[3] The strawberry extract agar consisted of 1,000 cc., of water, 15 gm. of agar and the expressed juice from 2 quarts of ripe strawberries. When titrated it was found to have a reaction of +31, Fuller's scale.

TABLE III.—*Summary of measurements of oospores*

Class (in microns)	Number of spores in each class	Class (in microns)	Number of spores in each class
14	2	27	63
15	2	28	58
16	1	29	41
17	6	30	36
18	3	31	11
19	7	32	13
20	10	33	3
21	14	34	6
22	14	35	3
23	19		
24	16	Total	400
25	34	Mean	26.42
26	38	Mode	27.0

The above description and measurements are believed to establish it as a fact that the organism dealt with in this paper is *Phytophthora cactorum*, since they agree so closely with those already published for that fungus by other workers (*1*, *8*, *10*). The finding of both paragynous and amphigynous antheridia in cultures of the strawberry Phytophthora is still further evidence that the organism is *P. cactorum* since Lafferty and Pethybridge (*3*) have reported the discovery of both kinds of antheridia in cultures of *P. cactorum* obtained by them from various sources.

INOCULATION EXPERIMENTS

Two methods of inoculation were used, one of them in the laboratory at the University of Chicago, the other in the field laboratory at Beebe, Ark. Procedure in the former was as follows:

Berries apparently free from disease were selected either from quart boxes purchased on the market (out of crates stamped "Klondike," "Haverland" or "Dunlap") or were picked from a small patch of Progressive, an everbearing variety, to which the writer had access. These berries were washed three or four times with sterile water, placed in sterile glass moist chambers in lots of 20 to 25 and inoculated by forcing a small fragment of mycelial growth from a pure culture down into the flesh. They were then covered up, but in such a way as to allow some ventilation, since it was found that too close covering kept the berries so moist that Botrytis on northern-grown berries and Rhizopus on berries from all sections frequently caused excessive decay before Phytophthora got well started.

Within three days, at 20 to 25° C., berries inoculated by this method, if they had escaped Rhizopus (or Botrytis), showed vascular browning and slight softening; within four days they showed a marked browning of the surface and had reached the tough leathery condition described earlier in this paper. For some unknown reason they did not have quite the appearance of berries naturally infected in the field, though they always gave Phytophthora when plantings were made from them, under aseptic conditions, into agar tubes. They were atypical externally, in that they did not show the color pattern or color transition, from pale yellow to brown to purple to red.

The same is true of berries inoculated by the second method, which, nevertheless, gave practically 100 per cent infection by Phytophthora. These berries (varieties Klondike and Aroma) selected as before as free or apparently free of disease, were washed with sterile water as soon as they were brought in from the field; 20 to 25 of them were then placed on clean, but not sterilized, newspaper, in about a quarter of an inch of water in pans, with five or six moldy "water-soaked" (leather rot) berries scattered among them. The experiment was al-

lowed to stand for an hour, with the berries exposed to the light; that is, for the length of time, and under the conditions, which make possible the discharge and germination of zoospores. The healthy berries were then removed, placed on clean moist newspaper in a sterilized pan and covered with another pan. Within three days the few that were taken out and cut showed softening and vascular browning; after one or two days more the remaining berries were covered with a scant growth of white mold and plantings from them yielded only Phytophthora.

In other words, the berries became infected under conditions in which infection by zoospores was not only possible but actually much more probable than infection, for example, by floating bits of mycelium. It is true, as noted earlier, that no zoospores were seen in any of the mounts made from Arkansas material. Yet, in the absence of proof to the contrary, this failure to find zoospores must be ascribed to a merely accidental failure to find conidia that were ripe for discharge.

From these experiments (second method) and from control experiments conducted in the same way, except for the diseased berries, it was necessary at the end of 24 hours to remove a few fruits that showed signs of leather rot, the result of having to use berries from a section of the country in which the rot was so common that it was likely to occur in incipient stages even in fruit selected with the utmost care. The remaining control fruits and controls wounded but not inoculated with mycelium (Chicago experiments) failed to give Phytophthora at any time, though by the end of five or six days 75 to 90 per cent of them were plainly diseased. Plantings from these diseased control fruits gave Pezizella, Sphaeronemella, an unidentified Rhizoctonia, and Botrytis. In experiments conducted in the field laboratory, Rhizopus gave very little trouble, probably because the berries came direct from the field and had been bruised only slightly or not at all.

Attempts to inoculate strawberries with Phytophthora by laying bits of an agar culture of the fungus on the surface of uninjured fruits were always unsuccessful.

INFECTION IN THE FIELD

Nothing definite is known about how infection takes place in the field. It may be that under field conditions the mycelium of Phytophthora is better able to enter uninjured fruits than it has appeared to be in the laboratory. It is certain that except after severe storms or general insect attack very few berries in the field have skin breaks of any sort, not to mention such wounds as were made in berries used for inoculation experiments. But conditions in the field which favor leather rot are precisely those which in the laboratory favor the discharge and germination of zoospores. It seems likely therefore that most of the field infection is caused by zoospores; whether all of it is so caused is matter for further investigation. Berries which touch the ground are always first affected, or show the disease first, on the lower side; it is not known, however, whether they become infected by zoospores or by direct growth of mycelium from the soil. This statement, in turn, raises the question whether the Phytophthora dealt with in this paper is a true soil fungus, in strawberry fields, or whether it lives on débris on the soil surface. Berries which hang free in the air and which may show the disease either at the tip or anywhere on the sides, could have become infected by zoospores or mycelium spattered onto them during rains; they might equally as well have become infected by means of zoospores which moved from the soil along the fruit stem to the fruit through the surface film of water which, during wet weather, covers all parts of the plant for hours at a time. More investigation is needed on all of these points.

Investigation is also needed on the relation of the calyx to rot of the fruit. Even under conditions of moderate rainfall and temperature there are always

to be found in the strawberry districts of the South, a few berries on which the calyces are discolored (brown or gray) and plainly diseased. In warm, wet weather the condition may become quite general. All varieties may show it but Klondike usually suffers most severely. Observations, both in the field and on the market, during the seasons of 1922 and 1923, have shown that diseased calyces are often associated with disease of the berry itself. Many berries have been found which showed signs of Phytophthora infection at the place where an apparently diseased calyx lobe lay in contact with the fruit surface. In such specimens a cross or longitudinal section always disclosed the typical vascular browning; plantings from affected areas always developed Phytophthora.

Many other fruits, on which the whole calyx was diseased, showed infection at the top in a ring or zone surrounding the base of the calyx. Plantings from this infected zone, in different berries, gave Phytophthora, Rhizoctonia sp. or Pezizella, or sometimes only bacteria. No cultures have been made from diseased calyces nor is there any proof that infection actually spreads from the calyx down into the fruit. The presumption is strong, however, that it very often does, or in other words, that the calyx when diseased is a source of danger to the fruit. One is reminded in this connection of the much more striking case of stem-end rot of citrus fruit, which, according to Winston, Fulton, and Bowman, (*17*) is probably due almost entirely to growth of either Diplodia or Phomopsis from the diseased calyx into underlying tissues of the fruit.

SUSCEPTIBLE VARIETIES

Under field conditions leather rot has been seen mainly on the varieties Klondike and Aroma, and always is much more serious on the former than on the latter. Gandy, Missionary, and Lady Thompson appear to be quite resistant if not immune. Klondike is the sole commercial variety in Louisiana and one of two, Aroma being the other, in Arkansas, Kentucky, and Tennessee; Aroma is the main variety in southwest Missouri (Monett-Neosho district) and is of considerable importance in southern Illinois. Gandy is grown commercially in southern Illinois, and Lady Thompson to a small extent in Arkansas and Tennessee. In the States discussed in this paper, the variety Missionary is grown very little and was actually seen in only one field in White County, Ark.

RANGE OF THE DISEASE

Leather rot was first observed by the writer on Klondike strawberries at Judsonia, Ark., during the first week of May, 1922, and again a few days afterward at Humboldt, Tenn. During the last week of May and the first two weeks of June it was found on the market in Klondike and Aroma strawberries from Bald Knob, Ark., from Monett, Mo., and from a small producing section near St. Louis, Mo. During the strawberry season of 1923 it was seen in the field at Gulfport, Miss., Amite and Tickfaw, La. (in the Hammond district), and at Beebe and McRae, Ark.; on the market (after June 1) it was seen in berries from southern Illinois and the Bowling Green section of Kentucky. Judsonia, Bald Knob, Beebe, and McRae, Ark., are all in White County and there is every reason to believe that the disease could have been found in practically all Klondike and Aroma strawberry fields in the county if there had been time to make the survey. It probably occurs also throughout the strawberry districts of west Tennessee, and not merely in the district around Humboldt. The writer has neither visited producing sections of east Tennessee nor seen strawberries from there but on the basis of information furnished by E. E. Conklin, supervising inspector for the United States Department of Agriculture at Cleveland, Tenn., during the strawberry shipping season of 1923, he is inclined to believe that the disease occurs in east Tennessee also. It is possible that the "lilac soft rot" reported by

Sherbakoff (*12*) from Tennessee is identical with leather rot, although Sherbakoff states that isolations from this "soft rot" gave a Pythium. The records for Monett, Mo., and for southern Illinois are based on one carload from each State, the only carloads from those States in which the rot was seen either in 1922 or 1923. This of course does not indicate heavy loss. There is other evidence also that in those States the disease is much less serious than in States farther south. But even if only the authentic records of occurrence are considered it is apparent that leather rot is rather widely distributed through the South in States which in 1922 (see fig. 5 and Table IV) were the leaders in strawberry production in the United States. Its relation to production and marketing of the crop will be brought out in the subsequent discussion.

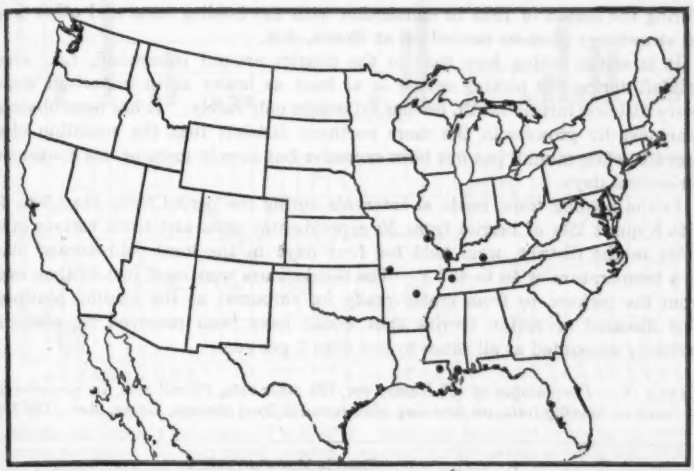

FIG. 5.—Distribution of leather rot of strawberries in the United States as determined during the picking seasons of 1922 and 1923 by inspection of fruit in the field and of shipped fruit on the market. The States and counties represented are as follows: Louisiana, Tangipahoa County; Mississippi, Harrison County; Arkansas, White County; Tennessee, Gibson, Bells, and Crockett Counties; Missouri, Newton, Barry, and Jefferson Counties; Kentucky, Warren County; Illinois, Union and Pulaski Counties

TABLE IV.—*Carlot shipments of strawberries by States of origin, 1922* [1]

Tennessee	3,592	Kentucky	756
Arkansas	2,190	Michigan	650
Missouri	2,043	Alabama	459
Virginia	1,670	New York	330
Maryland	1,629	Florida	325
Louisiana	1,540	New Jersey	274
North Carolina	1,101	Illinois	260
Delaware	940	All other States	789

RELATION OF LEATHER ROT TO RAINFALL

Strawberry growers in the region dealt with in this paper have observed that "water soak" (leather rot) is worse during wet weather than during dry weather. Their use of the term implies a belief, often explicitly stated, that the berries

[1] Figures taken from the Yearbook of the United States Department of Agriculture for 1922 (*14*, *p. 778*)

simply take up too much water and become worthless. As a matter of fact this probably does happen with some of the fruit, during wet weather. It is well known that strawberries produced during such weather are likely to be soft, succulent, and easily damaged even by the most careful handling. It is entirely possible that the local name of "water soak" is sometimes applied to berries that are not infected by any parasite but have merely been rendered soft and mushy by too much rain. It has been shown, however, that much of the condition known to the growers as "water soak" is actually the disease caused by Phytophthora. On the other hand, and in full agreement with observations by the growers, it has been found that there is a close relation between leather rot and rainfall. The fact that this relation exists was established in a general way by the writer, in 1922, and more definitely, though with qualifications, during the season of 1923 in connection with the holding tests and other work on strawberry diseases carried on at Beebe, Ark.

It is worth noting here that in the district around Hammond, La., where rainfall during the picking season is at least as heavy as in important strawberry districts farther north, leather rot occurs only rarely. It has been observed moreover by growers in the more northern districts that the condition often prevails when rainfall has not been excessive but merely more or less continuous for several days.

In the holding tests, made at intervals during the period from May 5 to 31, 2 to 8 quart lots of berries from 30 experimental plots and from various other fields in the district, were held for four days in the local cold-storage plant at a temperature of 40 to 45° F. The filled quarts were used just as they came from the pickers, or from crates ready for shipment at the loading platform. The diseased or rotten berries that would have been removed by resorting certainly amounted at all times to less than 1 per cent.

TABLE V.—*Percentages of (1) leather rot, (2) other rots, (3) all rots, in strawberries used in holding tests, on first day after removal from storage, Beebe, Ark., 1923*

Kind of rot	Dates in May when picked—													
	5	7	8	9	11	14	15	16	17	18	19	21	23	26
Leather rot	1.3	0.0	3.1	3.0	41.5	4.0	5.1	29.2	45.4	68.5	10.9	6.2	16.1	25.1
Other rots	5.0	7.2	2.2	6.5	11.7	.2	2.5	.9	3.3	.0	.4	2.9	31.7	27.5
All rots	6.3	7.2	5.3	9.5	53.2	4.2	7.6	30.1	48.7	68.5	11.3	9.1	47.8	52.6

At the end of the four days the various lots were removed and the berries were carefully hand sorted and counted to determine the amount and kind of rot present. Except where noted, the count was made immediately after the fruit was removed from cold storage. A summary of results obtained in these tests is given in Table V. The various rots were determined by touch and sight, these being checked occasionally by cutting diseased fruits or making cultures from them. Figure 6 is based on these counts and shows graphically the relation of rot to weather conditions, particularly rainfall. The rainfall record was obtained by means of a gauge loaned by the United States Weather Bureau at Little Rock. Since there was no accurate local record, the temperature data used are those recorded by the Weather Bureau (*15*) for Little Rock, Ark., which is about 35 miles from Beebe and considerably nearer to it than are any of the other weather stations in that part of the State.

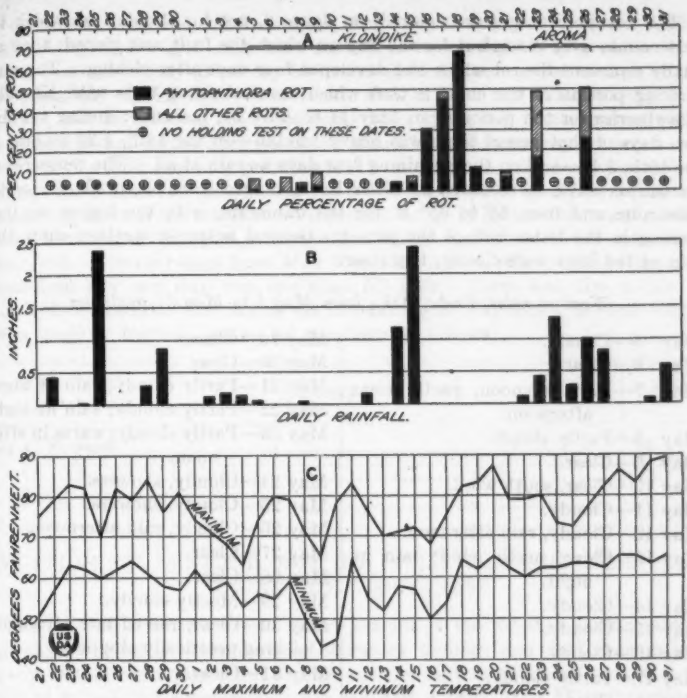

FIG. 6.—A. Percentage of rot found immediately after removal from storage ,in various lots of strawberries held for four days at a temperature of 40 to 45° F. Percentages are entered for the day on which the berries were picked. B. Daily rainfall in inches. Record of rainfall was kept at Beebe, Ark., by means of a standard rain gauge loaned by the United States Weather Bureau. C Daily maximum and minimum temperatures, as recorded by the United States Weather Bureau at Little Rock, Ark., about 35 miles from Beebe.

TABLE VI.—*Daily temperatures, April 21 to May 31, Little Rock, Ark., and rainfall for the same period, at Beebe, Ark.*

Date	Temperature (degrees F.)		Rainfall	Date	Temperature (degrees F.)		Rainfall
	Maximum	Minimum			Maximum	Minimum	
			Inches				*Inches*
Apr. 21	75	51	0.41	May 12	76	55	0.15
22	79	57	.00	13	70	52	.01
23	83	63	.01	14	71	58	1.20
24	82	62	1.15	15	72	57	2.40
25	70	60	.00	16	68	50	.00
26	82	57	.00	17	75	54	.00
27	79	64	.30	18	82	64	.00
28	84	61	2.38	19	83	62	.00
29	76	58	.86	20	87	65	.00
30	81	57	.00	21	79	62	.00
May 1	74	62	.00	22	78	60	.11
2	64	57	.13	23	79	62	.47
3	68	59	.18	24	73	62	1.33
4	63	53	.15	25	72	63	.27
5	74	56	.06	26	77	63	1.00
6	80	55	.00	27	82	62	.80
7	79	59	.00	28	86	65	.00
8	71	50	.06	29	83	65	.00
9	65	43	.00	30	88	63	.08
10	78	45	.00	31	89	65	.88
11	83	64	.00				

The rot percentages are entered, on figure 6, not for the day on which the first counts were made but for the day on which the fruit was picked; they actually represent the rot which had developed four days after picking. The most striking portion of the chart is that which covers holding tests with Klondike strawberries for the period from May 14 to May 21, inclusive; during the first two days of that period there was heavy rainfall—on the 14th, 1.20 inches; on the 15th, 2.40—and on the remaining four days no rain at all. The temperature for the six days, as recorded at Little Rock, ranged from 68 to 83° F. for the maximum and from 50 to 65° F. for the minimum, with the higher readings coming in the latter half of the period. General notes on weather show that four of the days were cloudy, two clear.

Weather notes, Beebe, Ark., from May 5 to May 31, inclusive

May 5—Cloudy.
May 6—Clear.
May 7—Rain forenoon, partly clear afternoon.
May 8—Partly cloudy.
May 9—Clear.
May 10—Clear, south wind.
May 11—Cloudy.
May 12—Cloudy, rain afternoon.
May 13—Clear; north wind; rain at night.
May 14—Cloudy.
May 15—Cloudy.
May 16—Cloudy.
May 17—Partly cloudy.
May 18—Clear.
May 19—Clear.
May 20—Clear.
May 21—Partly cloudy; rain at night.
May 22—Partly cloudy; rain at night.
May 23—Partly cloudy; warm in afternoon.
May 24—Cloudy, showers.
May 25—Cloudy, showers.
May 26—Cloudy, rain afternoon.
May 27—Clear.
May 28—Clear.
May 29—Mostly cloudy.
May 30—Clear, rained late afternoon; picking practically stopped.
May 31—Clear.

It is apparent that during these six days there was excellent opportunity for determining what correlation, if any, exists between rainfall and leather rot of strawberries. Reference to the chart shows that in this instance positive correlation does exist. For fruit picked on the 14th and 15th, the days when the rain occurred, the percentage of rot was about what it had been during the previous 10 days. (This statement is based partly on what shows on the chart and partly on field observations and counts.) But for the fruit picked on the 16th and counted four days later the percentage of rot rose to 29.2 per cent, on the 17th to 45.4 per cent, and on the 18th, four days after the first rain, to a maximum of 68.5 per cent; on the 19th it dropped back to 10.9 per cent and on the 21st to 6.2 per cent. (Compare 3 per cent on the 9th and 4 per cent on the 15th.) This course of events is practically that observed in inoculation experiments, where the first visible symptoms of leather rot appeared on the third day after inoculation and fully developed rot on the fourth. It also helps to explain reports by representatives of the Federal Market News Service, by receivers on the markets, and by the sales manager of the local association at Beebe that Klondike berries picked and shipped on the 18th showed more loss on arrival at market than did those shipped on any other day from the 14th to the 21st.

The marked drop in rot from 68.5 per cent on the 18th to 10.9 on the 19th and 6.2 on the 21st is best explained by assuming that very little infection occurred after the 15th, the last day of rainy weather. It is exactly what should be expected if Phytophthora infection occurs only in wet weather and takes three to four days to become visible rot. If, however, there had been rains for several days or only two or three days apart, the attacks would have so overlapped as

to obscure the rise and fall in amount of rot due to any one of them. Under such conditions it might easily have seemed, as it actually has to growers many times, that rot on any given day had resulted from the weather of that or the preceding day. When the course of one attack, as in the instance under discussion, can be followed from beginning to end, the lag in occurrence of the maximum as well as the duration of the attack both become very apparent.

Under this topic one more point remains to be covered. As mentioned earlier, growers have observed that leather rot often occurs when rainfall is not heavy but merely more or less continuous for several days. It is therefore worth noting that the rot did occur in just such weather during the course of the work here described. The four days from May 2 to May 5 were rainy, but the heaviest rainfall on any one day was less than 0.2 inch. There was also a rain of 0.06 inch on the 8th. Yet during the five days from May 5 to 9, the first counts being made on the 5th, leather rot was found as follows:

	In the field	In holding tests
May 5	No counts	1.3.
6	do	No counts.
7	do	0.0.
8	8.0	3.1.
9	5.0	3.0.

RELATION OF LEATHER ROT TO FIELD TEMPERATURES AND DEGREE OF CLOUDINESS

The relation of leather rot to other elements of the weather besides rainfall can not be so easily determined. Laboratory findings and field observations justify certain assumptions but are not yet complete or extensive enough to be used as a basis for conclusions. For the period from May 14 to 21 general cloudiness and fairly low temperatures during the first part of the period prolonged the time during which both soil and plants were covered with a film of moisture. This, in turn, by favoring the release and distribution of zoospores, would presumably increase the amount of rot and lengthen the time during which the rot would be heavy. But on the other hand, higher temperatures during the latter part of the period would hasten development of the rot in infected berries and so bring the attack to an end sooner, provided there was no further rainfall. They might thus account, in part at least, for the sharp drop from 68.5 per cent of rot on the 18th to 10.9 per cent on the 19th. It is a fact, noted both by the writer and by many growers, that during hot weather three or four days after a rain leather rot develops and becomes visible very rapidly. On the 18th and again on the 26th, 28th, and 29th, all of which had maximum temperatures of 77 to 85° F., numerous packed crates were seen in which the berries picked in the morning as being apparently sound had by afternoon developed so much visible Phytophthora rot that they had to be thrown away.

PROPORTION OF LEATHER ROT TO OTHER ROTS

IN HOLDING TESTS.—During the six-day period mentioned above, the percentage of rots other than leather rot (mostly Pezizella and Rhizoctonia sp.) in cold storage lots of Klondike berries, showed no progressive increase from day to day. In fact, on the 18th when leather rot was the highest, no other rots were found. During the period from May 5 to 14 there was no progressive increase in any of the rots, probably because rainfall was light and percentage of all rots low. On the other hand, there is evidence, furnished, it is true, by only two holding tests

(fig. 6), that on Aroma berries during the later period of rainy weather, May 22 to 27, leather rot increased after the rain and that as it increased other rots decreased. Owing to press of other work, the first count on the two tests had to be postponed until about 30 hours after removal of the fruit from storage. Percentage of all rots is therefore excessively high, and not strictly comparable with the percentage found when counts were made immediately after removal from storage. Reference to figure 6 and Table VIII, May 23 and 26, shows nevertheless that on both of these dates, rots other than that caused by Phytophthora constituted a much larger proportion of the total rot, on the variety Aroma, than they did during the preceding week on the variety Klondike. But according to evidence already adduced, the rainfall from May 22 to 27 was very favorable for the development of leather rot. The obvious explanation, and one which is supported by observations and counts made in the field, is that the variety Aroma is less susceptible than the variety Klondike to leather rot.

IN THE FIELD.—Incomplete data on occurrence of the various rots in the field are given in Table VIII. These figures indicate that under field conditions there is for both Aroma and Klondike, a progressive increase in Phytophthora rot as the season advances and a corresponding decrease in other rots; for Aroma, a preponderance of "other rots" over leather rot about as great as that noted in the holding tests.

TABLE VIII.—*Percentage of (1) leather rot, (2) other rots, occurring in strawberry fruits in fields, near Beebe, Ark., May, 1923.*[a]

Date	Leather rot	Other rots	Variety
May 8	19.1	80.9	Klondike.
May 9	26.6	73.4	Do.
May 15	88.4	11.6	Do.
May 16	91.2	8.8	Do.
May 25	14.8	85.2	Aroma.
May 26	35.0	65.0	Do.
May 28	55.0	45.0	Do.

[a] Percentages calculated on the basis of total rot as 100 per cent.

TABLE IX.—*Percentage of (1) sound, and (2) rotten strawberries in fields near Beebe, Ark., May, 1922*

Date	Sound	Rotten	Variety
	Per cent	*Per cent*	
May 8	62	38	Klondike.
May 9	82	18	Do.
May 16	90	10	Do.
May 18	76	24	Do.
May 25	60	40	Aroma.
May 26	25	75	Do.
May 29 [a]	33	67	Do.

[a] The figures for May 29 are the average of an estimate, by three competent observers, of the loss from rot on May 28 and 29 in the district immediately surrounding Beebe; all other figures in the table are based on field counts made on the date opposite which they are entered.

RELATION OF STRAWBERRY PHYTOPHTHORA TO TEMPERATURE IN LABORATORY EXPERIMENTS AND IN HOLDING TESTS

IN LABORATORY EXPERIMENTS.—The effect of various temperatures on the growth of the fungus (in culture) was tested by holding sets of three plates (of potato dextrose agar) inoculated with the organism, in each of nine compartments of an Altmann constant temperature apparatus. At the end of 10 days the plates were removed and the diameter of the growth was measured. Results of one such experiment gave the following results:

Average temperature °C.	1.0	3.6	8.2	11.8	12.6	14.7	15.4	17.6	19.1
Diameter of growth in mm.	0.0	0.0	3.5	4.4	5.6	6.2	6.9	7.5	8.0

The growth at room temperature, 20 to 25°C. averaged 8.6 for the 10 days. At 36°C. there was no growth at all. Evidently, therefore, the optimum for growth lies somewhere between 25 and 36°C. though no tests were run at these intermediate temperature, since they were not obtainable at the time the above test was made. The growth at 8.2°C. was a flat, dense surface mat; at higher temperatures the growth was more aerial and fluffy, both of these characters becoming more pronounced with each successive rise in temperature. At all temperatures where growth occurred the fungus grew both on the surface of and down into the agar.

IN HOLDING TESTS.—As already noted, the fruit used for holding tests was apparently healthy when stored. Yet after four days' storage at 40 to 45°F. all 14 lots of this fruit showed 4 per cent or more of rot (Table VIII) and six of them 25 to nearly 70 per cent. The rots found on the first day after removal from storage were caused by Phytophthora, Pezizella, Rhizoctonia sp. and Botrytis, with rot caused by Phytophthora greatly exceeding the other three in 11 of the Klondike lots and about equaling them in the other Klondike lot and two lots of Aroma. At temperatures between 40 and 45°F., all of the fungi named, but especially Phytophthora, were able to grow so rapidly that within four days they rendered a large part of the fruit worthless. Most of the infection was undoubtedly present though not apparent externally when the fruit was stored. The remainder must have developed through spread of Phytophthora from diseased to healthy berries. Evidence that this had happened was seen repeatedly during the making of counts, and later during the inspection of carload lots of strawberries on the Chicago market. The problem, however, is one which cannot be solved without further investigation.

CONTROL EXPERIMENTS

Strawberries used in the holding tests discussed above came from a number of fields near Beebe in which experiments had been conducted on control of fruit rots by spraying and dusting. The details of these experiments and the apparent effect of spraying and dusting on percentage of total rot have been reported (*13*). The data on leather rot are given in Table X, together with data for "other rots" and total rot, which are included for purposes of comparison.

TABLE X.—*Effect of spraying and of dusting on fruit rots of strawberries, at Beebe, Ark., 1923*

	Holding tests, per cent of rot four days after picking			
	Spray	Copper dust	Sulphur dust	Control.
KLONDIKE-THORNTON PLOTS				
Leather rot	22.5	24.3	0	34.8
Other rots	2.1	1.7	0	1.8
Total rot	24.6	26.0	0	36.6
KLONDIKE-ABINGTON PLOTS				
Leather Rot	16.3	16.8	0	18.0
Other rots	2.8	5.8	0	10.3
Total rot	19.1	22.6	0	28.3
AROMA-THORNTON PLOTS				
Leather rot	17.2	19.6	4.5	17.7
Other rots	21.9	40.4	37.0	42.9
Total rot	39.1	55.8	41.6	60.3

The results presented above indicate some control of leather rot, though not such as would be considered satisfactory from a commercial point of view. It should be remembered, however, that they were obtained during an extremely wet season, in fields known to have suffered heavily from rot the previous year. It is planned to repeat these experiments another year.

It is planned also to test the value of mulching as a means of preventing rot. Mulching is known to be good practice in other strawberry sections, and it proved to be quite effective during the season of 1923, in one field near Beebe, where it was tried out in a small way by a grower. The material used was pine needles, applied in such a way that when well settled they made a layer about an inch thick. The writer visited this field on several occasions, but failed each time to find rot of any kind in the mulched rows; in the unmulched rows alongside, leather rot and other rots were fully as common as in any of the unmulched fields in the Beebe district. No counts were made.

The scarcity of leather rot in the district around Hammond, La., has already been referred to; attention has also been called to the fact that this district has as heavy rainfall as the strawberry districts farther north. But in the Hammond district practically all strawberry fields are mulched. In the writer's opinion this is precisely the reason why the disease is so scarce there. It has been found, but only in a few unmulched or poorly mulched fields.

SUMMARY

1. A serious disease of strawberry fruits is reported which is apparently new to American phytopathological literature, though what seems to be the same disease was reported by Osterwalder (*5*) in 1912 as occurring in Switzerland.

2. It is demonstrated that the fungus, a Phytophthora, constantly associated with this disease is pathogenic to strawberry fruits. Evidence is presented which indicates that this Phytophthora is identical with *P. cactorum*, first described by Lebert and Cohn and reported in the United States as parasitic on apples, pears, ginseng and rhubarb.

3. The disease is described in detail and the common name of "leather rot" is suggested for it.

4. It is demonstrated by this investigation that there is a close relation between leather rot and rainfall, and that in the region studied, the rot can be expected to reach a maximum within three to four days after heavy rains. Temperature is important also but its effect can not be determined without further study.

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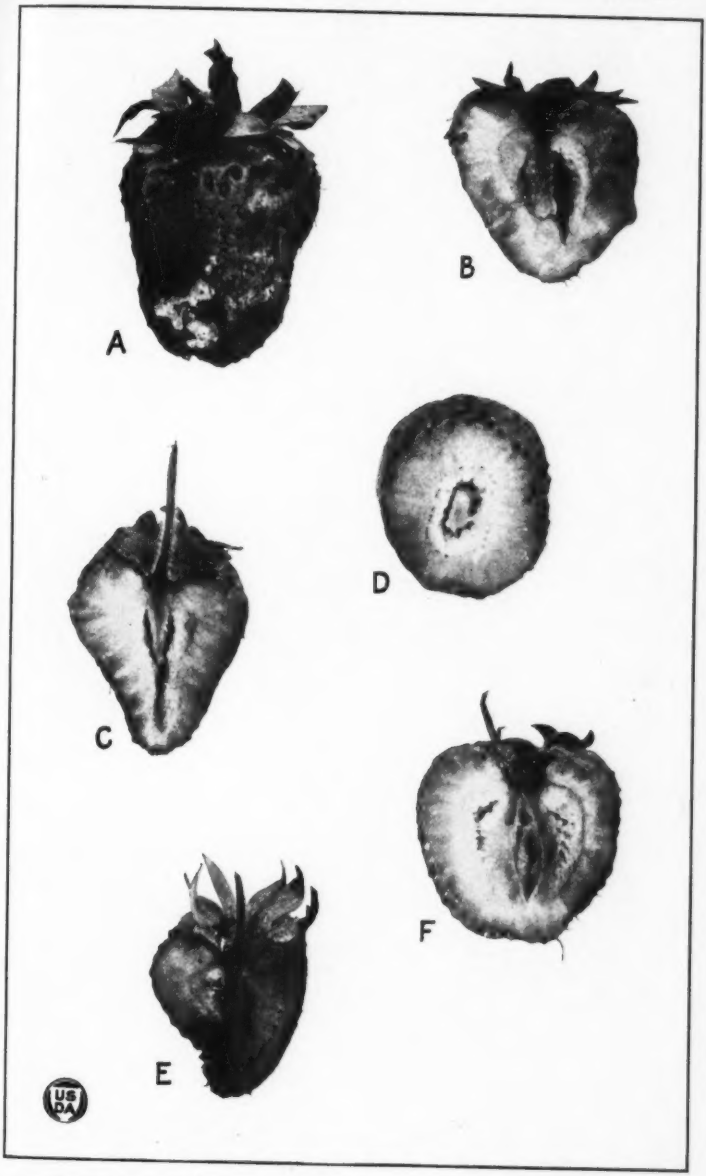
PLATE 1

Leather rot of strawberry caused by *Phytophthora cactorum* (Leb. et Cohn) Schroeter.

A.—External appearance of rotting strawberry showing patches of the white mycelium of the fungus.

B, C.—Sections of berries showing the "water-soak" condition.

D, E, F.—Sections showing characteristic browning of the vascular tissue.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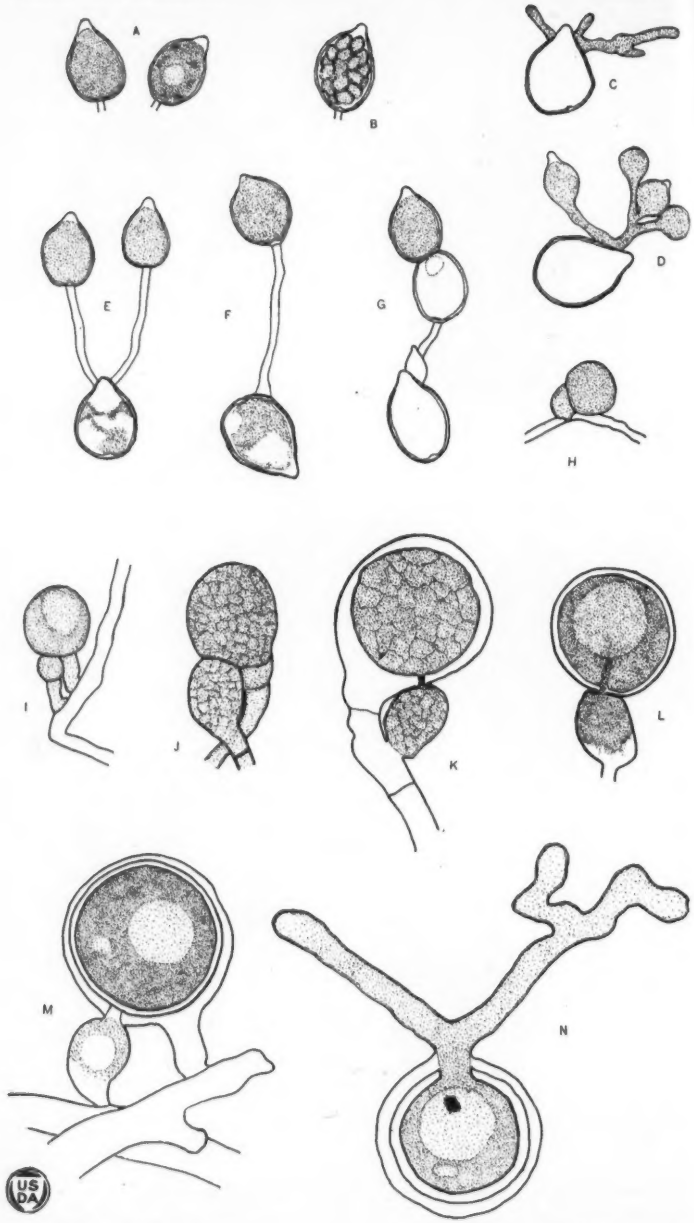
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PLATE 2

Leather rot of strawberry caused by *Photophthora cactorum* (Leb. et Cohn) Schroeter.

A, B.—Conidia.

C to G.—Secondary and tertiary conidia.

H to M.—*Paragunous antheridia.*

N.—Germinating oospore.

THE CAMBIUM CURCULIO, CONOTRACHELUS ANAGLYPTICUS SAY[1]

By FRED E. BROOKS, *Entomologist, Fruit Insect Investigations, Bureau of Entomology*, with detailed description of larva and pupa by R. T. COTTON, *Entomologist, Stored-Product Insect Investigations, Bureau of Entomology, United States Department of Agriculture*

INTRODUCTION

Orchardists in the eastern part of the United States who practice jarring plum and peach trees to capture the beetles of the plum curculio, *Conotrachelus nenuphar* Herbst, occasionally obtain with these beetles specimens of the nearly related species *Conotrachelus anaglypticus* Say. This beetle is slightly smaller and somewhat more active than the plum curculio but in a general way resembles it rather closely, although there is little difficulty in distinguishing between the two. The larva is found in a variety of situations, but a frequent place of feeding and development is in the cambium around wounds in the bark of various kinds of orchard and forest trees. On account of this feeding habit the common name "cambium curculio" is here applied to the insect.

In the year 1910 E. L. Jenne, working under the direction of Dr. A. L. Quaintance, in charge of Fruit Insect Investigations in the Bureau of Entomology, made careful observations on the egg-laying habits and larval development of this insect at Gainesville, Ga. Mr. Jenne observed the species as a possible enemy of the fruit of the peach. In 1922 Oliver I. Snapp, also of the office of Fruit Insect Investigations, made observations on the insect as an enemy of peaches, at Fort Valley, Ga. Data obtained by both Jenne and Snapp are included in this paper, together with those collected by the writer in West Virginia over a period of several years. The studies in West Virginia have revealed the insect as an habitual feeder under the bark of fruit and other trees.

Both Jenne and Snapp found the larvae of the cambium curculio feeding in peaches in company with the larvae of the plum curculio. This association in feeding of the two similar species makes it important that observers be able to distinguish between them while in the larval stage. Accordingly, R. T. Cotton, a specialist in curculionid larvae, who is employed under the direction of Dr. E. A. Back, Stored-Product Insect Investigations in the Bureau of Entomology, furnished upon request the drawings (Pls. 2, 3, and 4) and comparative descriptions that are used in this paper.

HISTORY AND DISTRIBUTION

This insect was named and described in 1831 by Thomas Say (*7, p. 18*).[2] Since the publication of the original description the species has been taken under a variety of conditions and in many localities. LeConte and Horn (*6, p. 234*), writing in 1876, record it from Massachusetts, Georgia, Kansas, and Texas. Blatchley and Leng (*1, p. 481*) add to its range the States of New Jersey, Michigan, Iowa, Illinois, Indiana, and Florida. The last named authors state that it is "abundant from New England to Michigan and Iowa, south to Florida and

[1] Received for publication April 22, 1924.

[2] Reference is made by number (italic) to "Literature cited," p. 380.

Texas." Scott and Fiske (*8, p. 34*) took it commonly in jarring peach and plum trees at Fort Valley, Ga. An unpublished note in the Bureau of Entomology shows that it was collected by a correspondent at Mount Solon, Va., in 1899. Felt (*3, p. 544*) records it as occurring at Poughkeepsie, N. Y., and in the Adirondack Mountains of the same State. It is recorded from the District of Columbia by Ulke (*9, p. 34, 55*), from southwestern Pennsylvania by Hamilton (*4, p. 376*), and from the vicinity of Cincinnati, Ohio, by Dury (*2*). In 1910 Jenne (*5*) collected it in numbers from peach trees at Gainesville, Ga., and in 1922 Snapp jarred numerous specimens from peach trees at Fort Valley, Ga. Data furnished by Thomas H. Jones, at the time entomologist of the Louisiana Agricultural Experiment Station, Baton Rouge, La., show that he reared adults of this species from larvae found at New Roads and Baker, La. The present writer has found the species abundant at French Creek, W. Va., and in other localities of that State. It would seem from the foregoing that the insect is distributed rather generally throughout much of the eastern and central portions of the United States.

FOOD HABITS

Very few references to the feeding habits of this species are published. Say (*7, p. 18*) says of it "Breeds in the fruit of hickory (Juglans)." Blatchley and Leng (*1, p. 480*) record taking the beetles from asters in Indiana and under moist bark and on various bushes in New Jersey. Felt (*3, p. 544*) states that beetles were taken on two successive days on a slippery elm tree that had been stripped of bark. This, he considered, indicated a certain attraction for this food plant.

Jenne, in his unpublished notes, speaks as follows of seven beetles that were jarred from peach trees at Gainesville, Ga.:

> The beetles were kept in a jelly glass with fresh peach leaves and fruit. No feeding was done on either fruit or foliage, except that they punctured the fruit at the receptacle of the stem. When the fruit was cut open the beetles would feed freely on it, but they would not puncture the skin.

In speaking of the larvae Jenne says, "In no case were they able to enter peaches except through a previously made break in the skin." From the 7 beetles referred to above Jenne reared 79 adults which "also refused to puncture the skin of either peaches or apples, but fed freely on these fruits when cut open."

In 1922 Snapp, while working with peach insects at Fort Valley, Ga., found that the beetles would oviposit in sound peaches. His unpublished notes state that 10 beetles were confined in a battery jar and supplied with peaches free from feeding or egg punctures. "The peaches were removed after they had been exposed to the beetles 48 hours and placed in wire cylinders. A number of eggs were deposited." The larvae from these eggs matured in the fruit and were permitted to enter the soil. Later a number of adults came through. With reference to these observations Snapp states, "The peach fruits exposed to the adults 48 hours, and in which eggs were deposited, were all sound peaches properly matured to that period in their development. Only peaches free from signs of any egg laying or feeding punctures were used for the purpose." He adds further, "I am of the opinion that each season a small proportion of the wormy peaches in Georgia is due to *anaglypticus*. Heretofore all Georgia wormy peaches have been attributed to the work of *nenuphar*."

Theo. Pergande, of the Bureau of Entomology, entered the following unpublished note in the bureau files: "May 24, 1899. Received from Jas. T. Clark, Mount Solon, Va., one specimen of this beetle (*Conotrachelus anaglypticus*), which he found, with several others which escaped him, in black knot on his plum trees, in which they had evidently been breeding."

April 27, 1923, Thos. H. Jones furnished the writer with data which show that he had reared beetles of this species from cotton bolls collected in two localities

in Louisiana. According to Mr. Jones the larvae in feeding in the bolls seem to prefer to work around the stem ends. He stated that the larvae were fairly common in the bolls but that he was unable to decide whether they cause primary injury or follow other injury.

The writer has made repeated unsuccessful attempts to rear larvae of the cambium curculio in sound peaches and apples. In these experiments the beetles deposited their eggs freely in the fuzz on the surface of peaches but the larvae on hatching did not enter the fruit. When the skin and flesh of peaches and apples were cut, however, the beetles oviposited in the wounds and the larvae developed successfully in the fruit.

Observations made over a period of several years in West Virginia, where this insect is abundant, indicate that the normal place for oviposition and larval development is around the borders of fresh wounds in the bark of many kinds of orchard and forest trees. The writer has captured beetles and found the larvae feeding at bark wounds in the following species of trees: Apple, *Malus sylvestris;* pear, *Pyrus communis;* pignut, *Hicoria glabra;* American hornbeam, *Carpinus caroliniana;* sweet birch, *Betula lenta;* American beech, *Fagus grandifolia;* American chestnut, *Castanea dentata;* white oak, *Quercus alba;* chestnut oak, *Q. montana;* red oak, *Q. borealis maxima;* tulip tree, *Liriodendron tulipifera;* service berry, *Amelanchier canadensis;* red maple, *Acer rubrum;* tupelo, *Nyssa sylvatica;* flowering dogwood, *Cornus florida;* and sourwood, *Ozydendrum arboreum.* The bark of many of these trees was scarified for the purpose of attracting the insects. Both larvae and beetles, however, were observed and collected under other conditions. They were found in great numbers in stumps and ends of logs cut in the forest for lumbering purposes, feeding about wounds in apple bark made by falling hailstones, and feeding in apple bark at edge of wounds made in pruning; larvae were found in wounds in dogwood bark made by boring larvae of *Aegeria* sp., in ax wounds made by lumbermen in trunks of tulip and dogwood trees, and in the edge of wounds in sweet birch made by boys in peeling bark for camp shelter; beetles were found hiding in fresh perforations made in pear bark by the yellow-bellied sapsucker, *Sphyrapicus varius varius;* were jarred from trees of peach, plum, and black walnut, *Juglans nigra,* and were found hiding in curled leaves on a hickory tree.

It is not unusual to find in June and July as many as a score of larvae feeding around a single wound in the bark of a tree trunk. In one case 24 larvae were taken from the edge of a wound in apple bark and in another 38 larvae were found under a small piece of bark at a wound in chestnut. Very little preference is shown for particular species of trees, although apple and pear seem to be especially attractive. Beetles, however, were attracted to every trapping place made in June, regardless of the species of tree used, and later the larvae were always present.

NATURE OF INJURY

Observations have shown that the cambium curculio is injurious in two distinct ways. It attacks the fruit of the peach and enlarges and prevents the healing of wounds in the bark of various kinds of trees (Pl. 1, C). While neither Jenne nor the writer found cases of injury to sound peaches, Snapp discovered that a small proportion of wormy peaches in Georgia is apparently due to this insect. There is also a possibility that sound cotton bolls are attacked and injured by the larvae, as has been pointed out by Jones. The larvae, in feeding in the bark, mine inward through the cambium from the edges of fresh wounds (Pl. 1, C). They do not mine extensively, but their feeding area, which is usually in the form of a band around the wound, varies from half an inch to 2 inches in width. Throughout this band all the cambium is likely to be destroyed and the wound enlarged to that extent. As the wound grows old the larvae cease to attack.

THE EGG

DESCRIPTION

The egg (Pl. 1, D) of the cambium curculio is somewhat variable in size and shape. Average specimens are oval, 0.7 mm. long by 0.4 to 0.45 mm. wide. The color is translucent white, with a yellowish tinge, the surface is slightly wrinkled, and the contents of the egg are plainly visible through the shell.

OVIPOSITION

Jenne states that eggs on peaches are deposited in the fuzz, the skin never being punctured. The writer observed that caged beetles would oviposit in the fuzz of peaches and on the downy surface of young apple shoots, but no eggs were found on the smooth skin of apple fruits nor on smooth apple bark. When fresh twigs were placed in the cages with slits cut in the bark the beetles would oviposit in the slits in preference to any other location. Whenever eggs were placed on a fuzzy surface the beetles would draw a little of the available fiber around the eggs. Many eggs were found partially embedded in the moist fiber of the inner bark around fresh wounds in trees. Jenne states that of 88 eggs observed all were laid in the night and 41 hatched in the night.

INCUBATION

At Gainesville, Ga., Jenne made observations on the incubation period of 188 eggs, the results of which are set forth in Table I.

TABLE I.—*Incubation period of 188 eggs of the cambium curculio at Gainesville, Ga., in 1910*

Number of eggs hatching	Duration of period
	Days
22	2
58	2.5
67	3
27	3.5
13	4
1	4.5

The average time required for hatching was 2.87 days. The first of the lot of 188 eggs was laid on May 20 and the last on July 10, oviposition therefore extending over a period of 52 days. Eggs laid early in the season were slightly slower in hatching than those laid late in the season. The 24 eggs laid in May required on an average 3.5 days to hatch, while the 13 laid in July required 2.79 days to hatch. The increasing warmth of the advancing season probably accounts for the shortening of the incubation period. Numerous eggs laid in West Virginia hatched in from 3 to 4 days. Jenne kept an egg record of seven beetles, but he does not state whether they were all females. The lot produced 188 eggs, an average of 26.86 each if they were all females.

THE LARVA

DESCRIPTION [3]

Mature larva of *Conotrachelus anaglypticus* (Pls. 2 and 3) from 7 to 9 mm. in length and from 1.5 to 2 mm. in width. Body white, fleshy, and legless, almost cylindrical but somewhat flattened ventrally. Ten abdominal segments, the ninth reduced and flattened, the tenth small and ventral.

[3] By R. T. Cotton.

Head light yellowish-brown, the anterior margin and mandibles much darker. Head, with labium and clypeus included, subquadrate, the width but slightly greater than the length. Width of head about 1.06 mm. Epicranial and frontal sutures distinct.

Frons subtriangular, with a dark median line indicating the carina extending about two-thirds of the distance from the posterior angle to the anterior margin of frons. Near anterior margin of frons on each side a group of two setae with a sensory spot between. Setae and sensory spot arranged in a straight, transverse line. In posterior angle of frons on each side two setae with sensory spot between arranged in a longitudinal curved line.

Clypeus broader than long, sides narrowing sharply to apical angles. Epistomal suture with two fine setae and an intermediary sensory spot on each side. Labrum subtriangular, broader than long, with three pairs of dorsal and six pairs of marginal setae.

Each epicranial half with thirteen setae and three sensory spots arranged as shown in Plate 2, H. One inconspicuous ocellus on each side. Antenna small, fleshy, two-jointed; basal joint with several small papillae.

Mandibles stout, subtriangular, slightly bifid at tip, excavated along the irregularly serrated cutting edge, no molar part, dorsal area with two setae.

Maxillary mala entire, tip obtuse, dorsal and ventral surfaces smooth. Dorsal surface with a longitudinal row of eight unbranched, stout setae. Ventral surface with four setae and a sensory spot near tip. Maxillary palp extending slightly beyond mala, two-jointed, borne by large membranous palpifer; proximal joint thick, cylindrical, bearing two sensory spots and with a small seta on the apical membrane; distal joint fingerlike, bearing several small terminal papillae. Three other large setae on maxilla, two of them near base of palpifer and the other about midway between palpus and end of cardo. A very minute seta with a sensory spot near stipes labii but usually concealed by the folded skin.

Mentum, submentum, and maxillary articulating area fused into a fleshy lobe. Three pairs of setae. Eulabium posteriorly enforced by a medium, triangularly bent chitinization. Between the palpi a small lobelike ligula. Labial palp short, conical, two-jointed; distal joint with several small terminal papillae. Eulabium bearing two small setae and two sensory spots on ventral surface. Ligula with two pairs of small setae and two sensory spots. Floor of buccal cavity composed of a fleshy hypopharynx laterally somewhat spinose and strengthened with small lateral chitinizations (not shown in figure). Hypopharyngeal bracon whitish and band-shaped, loosely connected with thin chitinizations laterally on hypopharynx.

Epipharynx carrying a pair of epipharyngeal rods. Between the rods two pairs of small, stout setae and a pair of three-lobed sensory spots. Prothorax dorsally not divided, thoracic plate lightly chitinized and provided with eight pairs of setae and two pairs of sensory spots. First thoracic spiracle pushed into prothorax but located rather far from anterior prothoracic margin; elongate, bifore, with the fingerlike air tubes pointing dorsad; at least twice as large as abdominal spiracles. Mesothoracic and metathoracic segments above divided into a spindle-shaped praescutum, a scuto-scutellum, and an alar area. Praescutum with one pair of setae, scuto-scutellum with four pairs, and each alar area with one seta.

Sternum of thorax consisting of the eusternum with the presternum depressed in front of it, a coxal lobe on each side, the sternellum, and the poststernellum. Eusternum of each thoracic segment bearing a pair of setae.

First seven abdominal segments divided into praescutum, scutum, scutellum, postscutellum, and alar area. Praescutum bearing a pair of setae, each scutellum five setae, and each alar area one pair of setae. Below the tergal areas is the epipleurum with two setae, the hypopleurum with two setae, the coxal lobe with one seta, and the eusternum with two pairs of setae. Eighth abdominal

segment dorsally with only three setae on the poorly defined scutellum. Ninth abdominal segment somewhat flattened dorsally and terminated with two pairs of long, stiff setae.

Spiracles of first eight abdominal segments bifore, the fingerlike air tubes pointing caudad.

COMPARISON OF LARVAE OF CONOTRACHELUS ANAGLYPTICUS AND C. NENUPHAR [4]

The larvae of *Conotrachelus anaglypticus* and *C. nenuphar* (Pls. 2 and 3) are, like all species of this genus, very similar in form and appearance. The full-grown larva of *C. anaglypticus* is, however, somewhat shorter and noticeably less robust than that of *C. nenuphar*. The following measurements indicate the relative difference in size between the average full-grown larvae of the two species:

C. anaglypticus: Length 7 to 9 mm.; width 1.5 to 2 mm.; dorso-ventral thickness 1.2 to 1.5 mm.

C. nenuphar: Length 8 to 10 mm.; width 2 to 2.5 mm.; dorso-ventral thickness 2 mm.

The larval head of *C. anaglypticus* is much flatter dorso-ventrally than that of *C. nenuphar* and the sides of the head are not so rounded as in *C. nenuphar*.

The most noticeable difference between the two species is in the frons. In *C. nenuphar* the dark median line or carina extends from the posterior angle to about the middle of the frons, whereas in *C. anaglypticus* the carina is much longer and extends fully two-thirds of the distance from the posterior angle to the anterior margin of the frons. Near the anterior margin of the frons there are on each side a group of two setae and a sensory spot. In *C. nenuphar* the setae and sensory spot of each group are arranged in the form of a triangle, but in *C. anaglypticus* the setae and sensory spot are arranged in a straight line.

HABITS AND LENGTH OF LARVAL LIFE

The white legless larva (Pl. 1, C, E, and F) of the cambium curculio is rather active and has a habit of throwing itself when disturbed, by straightening with a snap from a curved position. Jenne says, "Full-grown larvae have a habit of leaping when irritated. They do this by curling their body up so as to hook the anal segment under the head. The body is then snapped out straight, causing the larva to jump from 3 to 4 inches along a smooth surface, or to a height of about 1 inch." The writer noticed that when a piece of bark under which the larvae were feeding was removed the insects would one by one throw themselves out of sight in the way described.

Jenne reared 88 larvae in fallen peaches, plums, and apples, most of the number being reared from peaches. The duration of larval life in the fruit of these 88 larvae is shown in Table II.

TABLE II.—*Length of larval life of the cambium curculio in peaches at Gainesville, Ga., in 1910*

Period in fruit	Number of larvae	Period in fruit	Number of larvae	Period in fruit	Number of larvae
Days		*Days*		*Days*	
11	1	19	7	25	4
13	1	20	10	26	2
14	10	21	8	28	1
15	5	22	3	29	2
16	5	23	4	30	4
17	6	24	3	33	1
18	11				

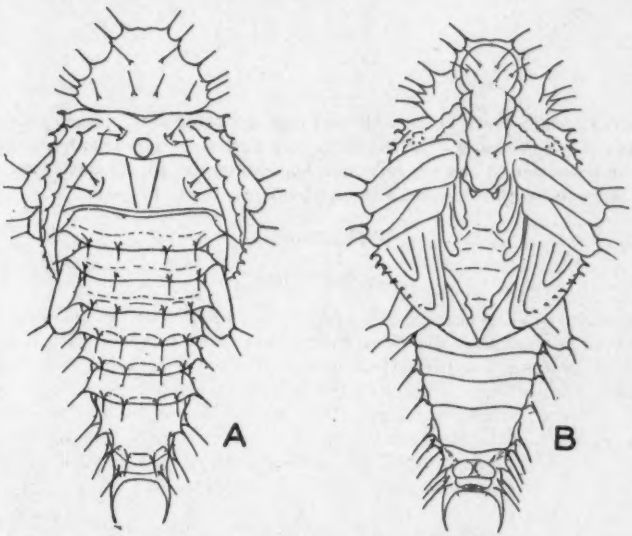
[4] By R. T. Cotton.

The insect in its active stages seems somewhat nocturnal in habits. Not only does oviposition take place principally at night, but 72 of the 88 larvae left their feeding places to enter the ground for pupation at night. The larvae burrow into the soil to a distance of from 1 to 3 inches to pupate; usually, however, pupation takes place about 1 inch beneath the surface of the soil.

THE PUPA

DESCRIPTION

Pupa of *Conotrachelus anaglypticus* (fig. 1) uniformly white in color; length about 5 to 5.5 mm. Head rounded, beak elongate and attaining base of mesothoracic legs; head with three pairs of setae borne on tubercles; beak with two pairs of setae borne on tubercles, one pair near base of antennae, the other pair between base of antennae and tip of beak. Prothorax subtriangular, rounded in front, with two pairs of antero-marginal setigerous tubercles, four pairs of latero-



Text fig. 1, A Text fig. 1, B.

FIG. 1.—*Conotrachelus anaglypticus:* A, Pupa, dorsal view; B, same, ventral view.

marginal, and three pairs of dorsal, setigerous tubercles. Mesonotum and metanotum each provided with two rather closely placed setigerous tubercles on each side of median area.

Abdomen with eight distinct tergites, median area of each tergite with a transverse row of four setigerous tubercles each of which has at base a minute seta; lateral areas of each tergite with a setigerous tubercle at base of which is a small seta. Ninth abdominal segment ventral, armed with a pair of stout, terminal spines.

Tips of wing pads attaining the fifth abdominal sternite; tips of metathoracic tarsi not extending to tips of wing pads. Elytra armed with longitudinal rows of short hooks or spines.

The pupa of *C. nenuphar* closely resembles that of *C. anaglypticus* but may be readily distinguished by its much larger size. Specimens forwarded to the writer vary in length from 6.5 to 8 mm.

LOCATION OF PUPA AND LENGTH OF UNDERGROUND PERIOD

The pupa (Pl. 1, F, and Pl. 4) occupies a smooth-walled cell within a spherical pellet of earth an inch or two below the surface of the ground. Jenne observed the time elapsing between the entering of the soil by the larvae and the emergence of the adults in 80 cases. Table III sets forth his data.

TABLE III.—*Number of days spent underground in the act of pupation by the cambium curculio at Gainesville, Ga., in 1910*

Number of insects	Period underground
	Days
1	17
13	18
13	19
25	20
18	21
6	22
1	23
2	24
1	29

Observations in West Virginia showed that a considerably longer period may be required in some cases. In one instance a number of larvae entered the soil under outdoor conditions on July 16. On September 15, 62 days later, live adults were still within the soil, although evidently ready to issue.

THE ADULT

DESCRIPTION

The adult cambium curculio (Pl. 1, A, B) is a small active snout-beetle from 3.5 to 5 mm. in length. The head, thorax, and under parts are dark brown to black and the legs and elytra reddish brown. The upper parts are clothed thinly with lighter colored hairs which form two narrow whitish lines on each side of the thorax, a broad, yellowish, oblique stripe at the base of each elytron, and a broad grayish band behind the middle of the elytra. The legs are marked, especially on the femora, with whitish bands of short hairs. The slightly curved snout is nearly as long as the head and thorax combined.

SEASONAL OCCURRENCE AND LONGEVITY

The beetles make their appearance from hibernation fairly early in the spring. Blatchley and Leng (*1, p. 481*) record taking specimens at Dunedin, Fla., on February 27 and March 17. Jenne captured specimens at Gainesville, Ga., April 16–30. Snapp began to secure beetles of this species in jarring peach trees at Fort Valley, Ga., on April 17. At French Creek, W. Va., the beetles appear by the middle of May and are present throughout the summer, activity being greatest during June and July. Two of the beetles which Jenne collected in April at Gainesville, Ga., were still alive on the 5th of the following November, another lived until September 11, and another until October 15. Jenne obtained beetles of the second generation from July 8 to August 9. Sixty-one of these young beetles were placed in an outdoor cage containing dry oak leaves on November 4 for the purpose of observing winter mortality. Of the lot placed in the cage at least 59 per cent were still alive on March 8 of the following spring.

LIFE CYCLE AND NUMBER OF GENERATIONS

Snapp found that in Georgia there are two generations of beetles annually; in West Virginia, on the other hand, there is but one.

Jenne recorded for 78 individuals the entire life cycle from the deposition of the eggs to the emergence of the adults from the ground. These records are given in Table IV.

TABLE IV.—*Time required by the cambium curculio for completing its life cycle at Gainesville, Ga., 1910*

Number of days	Number of insects	Number of days	Number of insects	Number of days	Number of insects
35	1	43	9	51	1
36	1	44	5	52	1
37	3	45	4	53	1
38	6	46	3	56	1
39	10	47	3		
40	3	48	5		
41	10	49	4		
42	7				

NATURAL ENEMIES

At French Creek, W. Va., the adult of a small hymenopterous parasite was found hovering around a larva of the cambium curculio under the bark of a tree. It was believed to be ovipositing in the body of the curculio larva. The specimen was captured and determined later by R. A. Cushman of the Bureau of Entomology as *Thersilochus conotracheli* Riley. Thomas H. Jones reports that he has reared from cotton bolls infested by larvae of this curculio from New Roads, La., specimens of a tachinid fly determined by Dr. J. M. Aldrich as *Myiophasia globosa* Townsend. Since both the species mentioned are known to parasitize Conotrachelus larvae, it is probable that they were attacking the larvae of *anaglypticus*.

METHODS OF CONTROL

Where this curculio attacks peaches there is little doubt that it will yield to jarring, spraying, and other control methods used against the plum curculio. In attacking the fruit the habits of the two species are so similar that separate treatments will not be called for. As a precaution against enlargement of bark wounds in fruit and other valuable trees by the larvae, the edges of fresh wounds made in the bark should be pared smooth and a heavy coat of white-lead paint, or some reliable tree paint, applied to all the injured parts. Such a coat of paint has been found effective in preventing the beetles from laying their eggs about the wounds.

SUMMARY

Conotrachelus anaglypticus is a rather common species from Massachusetts to Florida and its range extends as far west as Iowa, Kansas, and Texas.

The species feeds in a great variety of situations. The beetles frequently occur on peach trees in company with those of the plum curculio, and the larvae have been found attacking peaches, cotton bolls, and the cambium and inner bark of many kinds of fruit, shade, and forest trees. In attacking the cambium of trees they work around the edges of wounds, retarding the healing process and enlarging the injured areas.

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The delicate white eggs are mostly laid at night in situations where the larvae are to feed. Eggs hatch in from 2 to 4 days and a single beetle lays on an average from 25 to 30 eggs. The larvae usually feed from 2 to 4 weeks and then enter the ground to pupate. In late summer and autumn the beetles issue from the soil and soon thereafter hibernate, probably in litter on the ground. In Georgia there are two generations of beetles each year, but from West Virginia northward there is but one generation annually.

The species is probably attacked by at least two parasites. In West Virginia a hymenopterous species, *Thersilochus conotracheli* Riley, apparently attacks the larvae and in Louisiana a dipterous parasite, *Myiophasia globosa* Townsend, was reared from cotton bolls infested by the cambium curculio.

Where this insect attacks peaches it will probably yield to jarring, spraying, and other methods used against the plum curculio. As a precaution against injury to the cambium of valuable trees, the edges of any wounds in the bark should be promptly pared smooth and the injured areas treated to a coat of white-lead paint or some reliable tree paint.

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PLATE 1

Conotrachelus anaglypticus

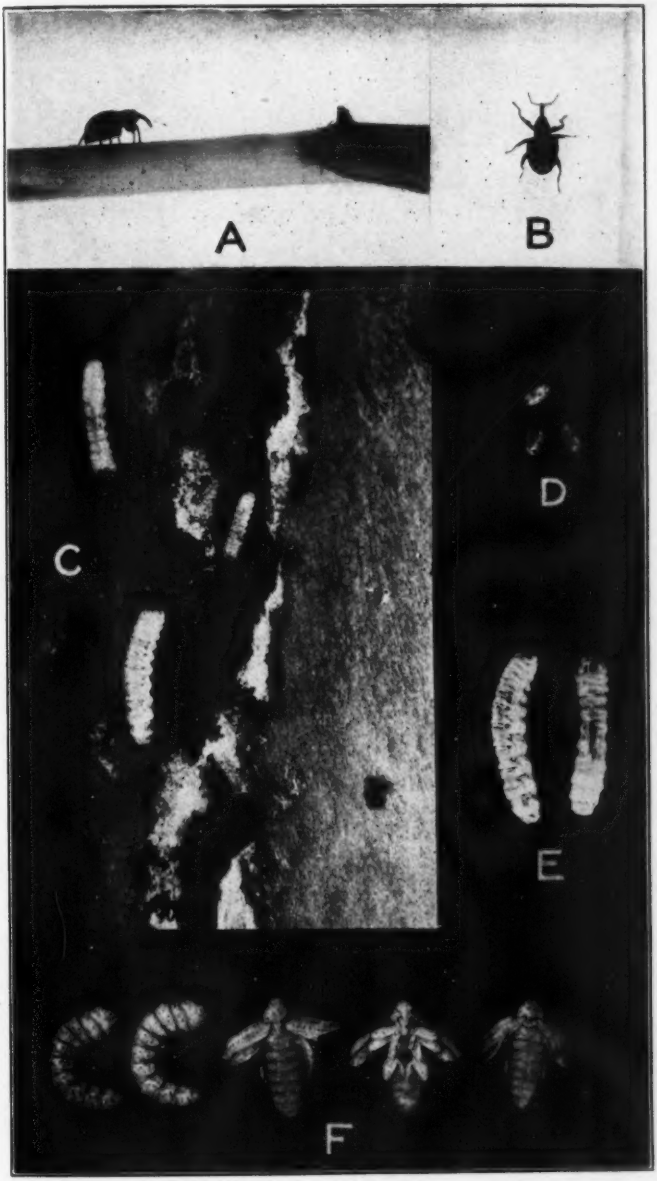
A, B.—Adult curculios. × 3

C.—Larvae mining into the live cambium from the dead wood on the right. × 3

D.—Eggs. × 4

E.—Larvae. × 3

F.—Larvae (left two) and pupae (right three). × 3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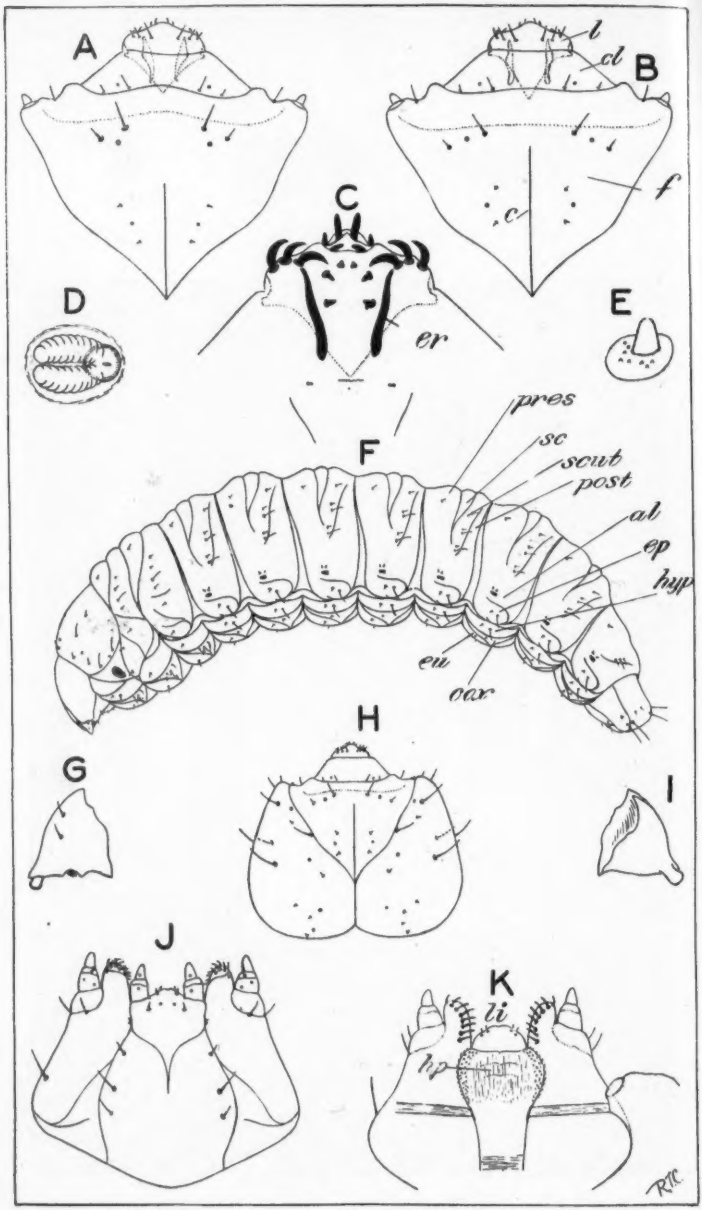
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PLATE 2

Conotrachelus nenuphar and *C. anaglypticus:* Details of larvae.

A.—*Conotrachelus nenuphar:* Anterior portions of face.

B.—*C. anaglypticus:* Anterior portions of face. *c.* Carina; *cl.* clypeus; *f*, frons; *l*, labrum.

C.—*C. anaglypticus:* Epipharynx. *er*, Epipharyngeal rod.

D.—*C. anaglypticus:* Thoracic spiracle.

E.—*C. anaglypticus:* Antenna.

F.—*C. anaglypticus:* Side view of larva. *al*, Alar area; *coz*, coxal lobe; *ep.* epipleurum; *eu*, eusternum; *hyp*, hypopleurum; *post*, postscutellum; *pres*, prescutum; *sc*, scutum, *scut*, scutellum.

G.—*C. anaglypticus:* Mandible, dorsal surface.

H.—*C. anaglypticus:* Front view of face.

I.—*C. anaglypticus:* Mandible, ventral surface.

J.—*C. anaglypticus:* Ventral mouth parts.

K.—*C. anaglypticus:* Dorsal view of maxillae, ligula, and hypopharynx. *li*, Ligula; *hp*, hypopharynx.

PLATE 3

Conotrachelus nenuphar and *C. anaglypticus:* Details of larvae.

A.—*Conotrachelus nenuphar:* Anterior portions of face.

B.—*C. anaglpyticus:* Anterior portions of face. *ant*, Antenna; *c*, carina; *cl*, clypeus; *em*, epistoma; *f*, frons; *l*, labrum.

C.—*C. nenuphar:* Epipharynx. *er*, Epipharyngeal rod.

D.—*C. nenuphar:* Mandible, dorsal view.

E.—*C. nenuphar:* Mandible, ventral view.

F.—*C. nenuphar:* Side view of larva. *al*, Alar area; *cox*, coxal lobe; *ep*, epipleurum; *eu*, eusternum; *hyp*, hypopleurum; *pres*, prescutum; *post*, postscutellum; *sc*, scutum; *scut*, scutellum.

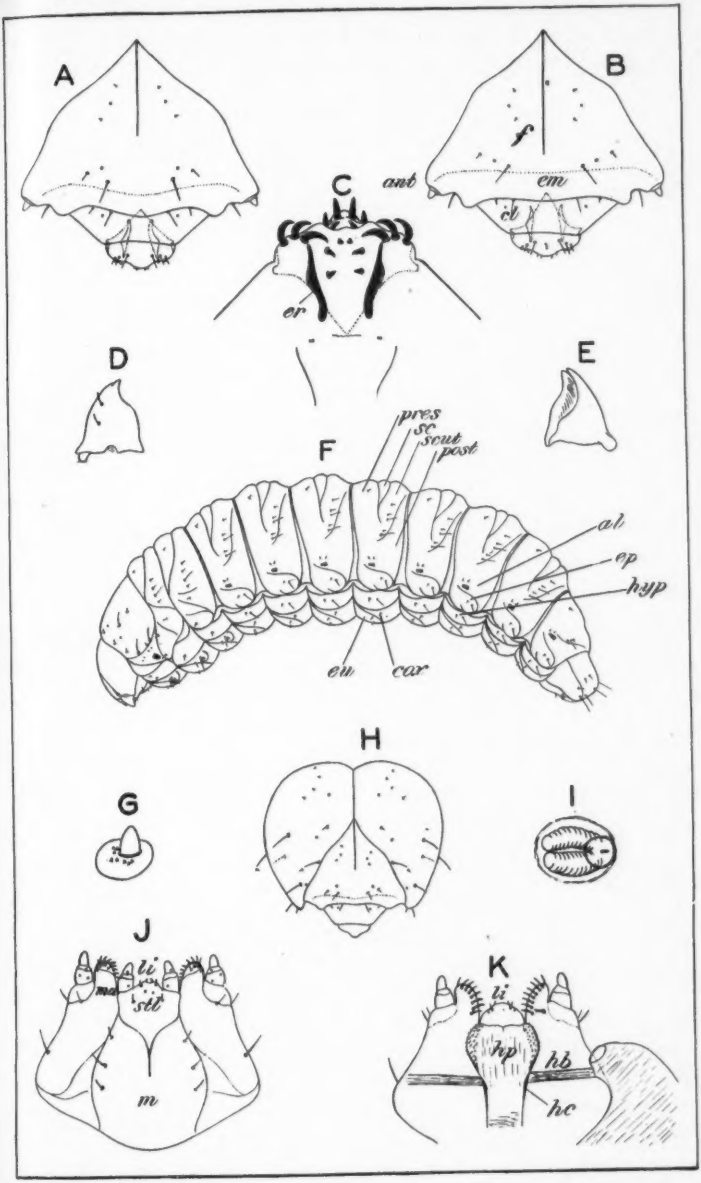
G.—*C. nenuphar:* Antenna.

H.—*C. nenuphar:* Front view of face.

I.—*C. nenuphar:* Thoracic spiracle.

J.—*C. nenuphar:* Ventral mouth parts.

K.—*C. nenuphar:* Dorsal surface of maxillae, ligula, and hypopharynx. *li*, Ligula, *hb*, hypopharyngeal bracon; *hc*, hypopharyngeal chitinization; *hp*, hypopharynx; *li*, ligula.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RELATION OF THE MOLECULAR PROPORTIONS IN THE NUTRIENT SOLUTION TO THE GROWTH OF WHEAT[1]

By M. C. SEWELL

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INTRODUCTION

The investigation herein reported was made to determine the comparative effects of three salts in varying proportions upon the growth of wheat in separate stages, namely, the seedling phase, the vegetative phase, and the fruiting phase. The salts used were potassium di-hydrogen phosphate, calcium nitrate, and magnesium sulphate. The problem was attempted with the expectation that possibly the data obtained would give some knowledge on which to base fertilizer practice pertaining to the wheat crop.

The results seem to show an interesting relation between the nitrogen and phosphorus potassium salts in the growth response. With a high molecular proportion of nitrogen, a maximum grain yield was obtained with a low molecular proportion of the phosphorus potassium salt. With a low molecular proportion of nitrogen, equally high grain yields were obtained only with a high proportion of the phosphorus potassium salt.

EXPERIMENTAL DATA

Wheat, first germinated in a nutrient solution, was grown in quartz sand, five plants to a culture. The containers were cylindrical half-gallon jars, glazed within and without, and holding 2,500 gm. of dry sand. After the seedlings were planted the jars were sealed with a wax preparation, and a paraffined paper cone was inserted as a supply orifice for adding water and the nutrient solutions. Surplus solution was removed by suction through a glass tube, plugged with glass wool at the lower end, which extended to the bottom of the jars. Measurements were made of transpiration losses, old solution removed, and new added every three and one-half days. During the fruiting phase distilled water was added daily between the three and one-half day periods.

The salts were used in 21 partial volume molecular proportions, all having a calculated osmotic value of approximately 1 atmosphere at 25° C. but differing (by increments of 1.8) in salt proportion.

The seedling phase consisted of a period of five weeks, the vegetative phase a period from the end of the seedling phase until the first appearance of flowering, and the reproductive phase extended from the vegetative phase until the maturity of the five best cultures of the group.

[1] Accepted for publication Dec. 15, 1923. Department contribution No. 150. The investigation reported in this paper was conducted in cooperation with the special committee on salt requirements of representative agricultural plants, National Research Council, Division of Biology and Agriculture. Dr. H. H. King, Department of Chemistry, Kansas Station, assisted in the experiment during the seedling phase of growth.

Five control cultures were used during each phase of growth studied. The control solution was Shive's [2] best solution, with a molecular proportion of 3.77 KH_2PO_4, 1.09 $Ca(NO_3)_2$, and 3.14 $MgSO_4$. This is a combination comparable to culture No. 16 in the accompanying figures.

In the study of the fruiting phase, cultures were begun January 2, 1923; the vegetative phase was marked as beginning February 6, and the fruiting phase, April 17. The plants were harvested June 1.

The daily maximum and minimum temperatures during these periods of growth are presented in Table I.

[2] SHIVE, J. W. A STUDY OF PHYSIOLOGICAL BALANCE FOR BUCKWHEAT GROWN IN THREE-SALT SOLUTIONS. N. J. Agr. Exp. Sta. Bul. 319, 63 p., illus. 1917.

TABLE I.—*Greenhouse temperatures averaged by five-day periods during the growth of wheat*

Date, 1921, 1922	Seedling phase		Date, 1922	Seedling phase for vegetative study		Date, 1923	Seedling phase for fruiting study		Date, 1922	Vegetative phase		Date, 1923	Vegetative phase for fruiting study		Date, 1923	Fruiting phase	
	Max.	Min.		Max.	Min.		Max.	Min.		Max.	Min.		Max.	Min.		Max.	Min.
	°F.	°F.		°F.	°F.		°F.	°F.		°F.	°F.		°F.	°F.		°F.	°F.
Dec. 6–10	82	50	Feb. 21–25	69	65	Jan. 1–5	72	55	Apr. 2–6	63	50	Feb. 10–14	76	65	Apr. 18–22	89	57
11–15	78	60	26–Mar. 2	64	59	6–10	74	59	7–11	64	61	15–19	79	63	23–27	94	60
16–20	82	56	Mar. 3–7	63	59	11–15	73	60	12–16	66	62	20–24	77	62	28–May 2	85	54
21–25	76	55	8–12	70	61	16–20	77	68	17–21	66	59	25–Mar. 1	80	67	May 3–7	87	57
26–30	75	65	13–17	65	62	21–25	73	62	22–26	66	59	Mar. 2–6	79	65	8–12	101	52
Jan. 1–5	75	56	18–22	70	63	26–30	73	62	27–May 1	69	62	6–11	83	63	13–17	93	45
6–10	74	62	23–27	67	60	31–Feb. 4	72	63	May 2–6	63	59	12–16	76	60	18–22	93	52
			28–Apr. 1	69	63	Feb. 5–9	72	61	7–11	68	63	17–21	75	60	23–27	105	54
									12–17	72	65	22–26	78	58			
									18–21	68	64	27–31	80	63			
												Apr. 1–5	83	63			
												6–10	80	61			
												11–16	90	60			

The 21 different molecular combinations of the three salts and the position of the cultures arranged in triangles are shown below. A duplicate triangle of cultures was conducted for each phase of growth. Since similar results were obtained in the duplicate cultures, the data from only one triangle for each phase of growth are presented here.

KH_2PO_4

21 *6–1–1*

19 *5–1–2* 20 *5–2–1*

16 *4–1–3* 17 *4–2–2* 18 *4–3–1*

12 *3–1–4* 13 *3–2–3* 14 *3–3–2* 15 *3–4–1*

7 *2–1–5* 8 *2–2–4* 9 *2–3–3* 10 *2–4–2* 11 *2–5–1*

1 *1–1–6* 2 *1–2–5* 3 *1–3–4* 4 *1–4–3* 5 *1–5–2* 6 *1–6–1*

$MgSO_4$ $Ca(NO_3)_2$

KH_2PO_4—$Ca(NO_3)_2$—$MgSO_4$

Control: 3.77 : 1.09 : 3.14

Salt nutrition of wheat. Molecular proportions of salts.

To visualize the data and to facilitate comparisons, figures 1, 2, 3, and 4 are given in lieu of tables. The sizes of the circles represent the comparative yields

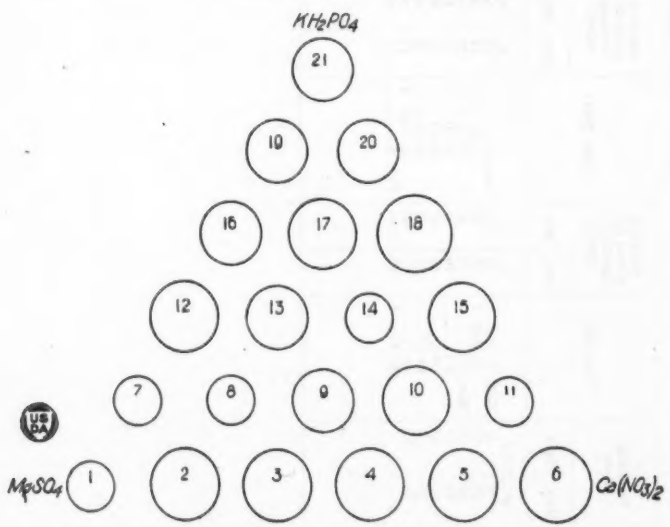

FIG. 1.—Salt nutrition of wheat: Seedling phase. Triangle 1, dry weight of tops.

measured in terms of dry weight of tops and, for the fruiting phase, dry weight of grain. The leaf colors of each of the various cultures during the seedling

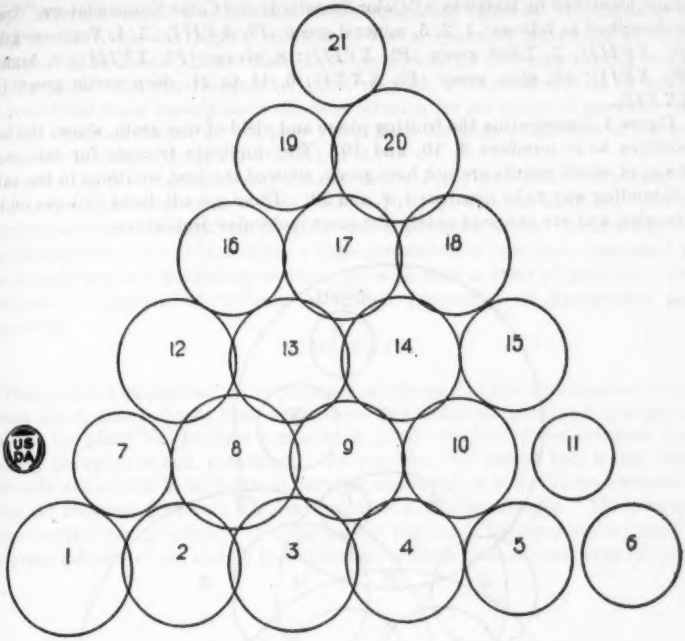

FIG. 2.—Salt nutrition of wheat: Vegetative phase. Triangle 1, dry weight of tops.

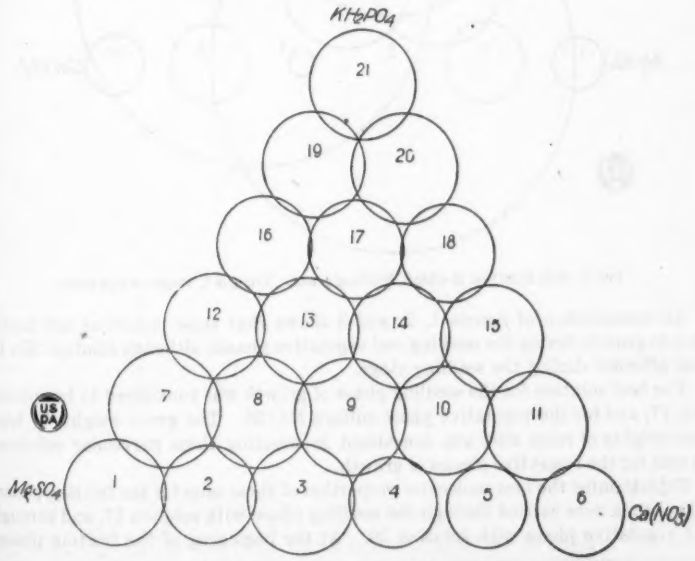

FIG. 3.—Salt nutrition of wheat: Fruiting phase. Triangle 1, dry weight of tops.

phase, identified by Ridgway's "Color Standards and Color Nomenclature," [3] may be described as follows: 1, 2, 5, mineral green (*Pl. XVIII*); 3, 4, Veronese green (*Pl. XVIII*); 7, Tiber green (*Pl. XVIII*); 8, rivage (*Pl. XVIII*); 9, lumiere (*Pl. XVII*); 10, glass green (*Pl. XXXI*); 6, 11 to 21, deep turtle green (*Pl. XXXII*).

Figure 4, representing the fruiting phase and yield of ripe grain, shows the best solutions to be numbers 3, 10, and 19. The duplicate triangle for this same phase, of which results are not here given, showed the best solutions in the same outstanding way to be numbers 4, 9, and 20. These are adjoining cultures on the triangles, and are hence of nearly the same molecular proportions.

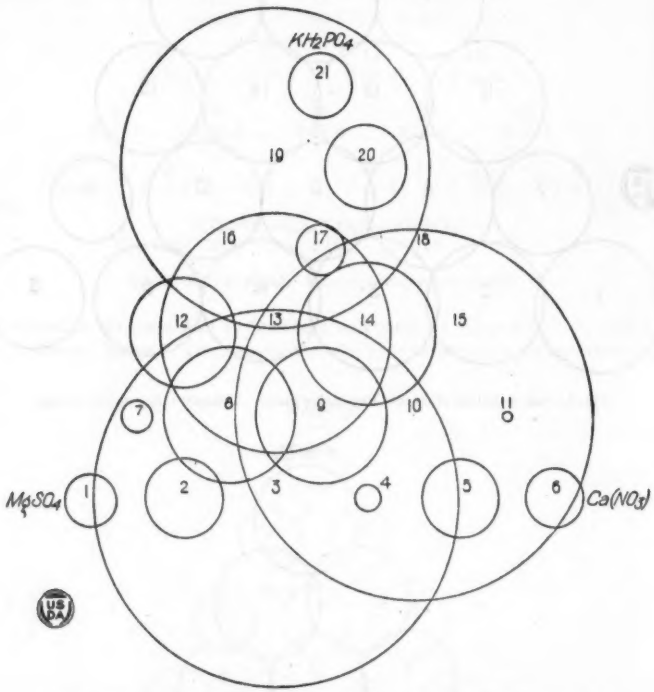

FIG. 4.—Salt nutrition of wheat: Fruiting phase. Triangle 1, weight of ripe grain.

An examination of figures 1, 2, and 3 shows that these solutions are favorable to growth during the seedling and vegetative phases, although solution No. 10 was off-color during the seedling stage.

The best solution for the seedling phase of growth was considered to be culture No. 17, and for the vegetative phase culture No. 20. The green weights of tops and weights of roots were also considered in selecting these particular solutions as best for the respective phases of growth.

To determine the best molecular proportion of these salts for the fruiting phase, all cultures were carried through the seedling phase with solution 17, and through the vegetative phase with solution 20. At the beginning of the fruiting phase,

[3] RIDGWAY, R., COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912.

the 21 different solutions were added to the designated cultures. In the order of KH_2PO_4, $Ca(NO_3)_2$ and $MgSO_4$, solution 17 had a molecular proportion of 4:2:2, and solution 20 one of 5:2:1. Since solutions 19 and 20 were outstanding in the fruiting phase from the viewpoint of yield of grain, a solution such as 20 might be considered from these results the best solution for all stages of growth. Al of these solutions named gave higher yields than the control solution of Shive's having the molecular proportion 3.77:1.09:3.14.

Considering the fruiting phase, since solutions 3 and 10 are so outstanding in producing a yield of grain as well as solution 19 or 20, and not markedly inferior to other solutions having high proportions of phosphorus and potassium, the fact is apparent that at maturity, a high proportion of nitrogen, compared to the phosphorus and potassium carriers, gives as high a yield of grain as a low molecular proportion of nitrogen and high proportion of phosphorus and potassium.

SUMMARY

These results emphasize the importance of nitrogen in the development of the wheat plant, and indicate that when there is a sufficient supply of nitrogen in the soil the plant will produce a maximum yield with less of the elements containing phosphorus and potassium. In practice, this means that where these elements are known to be deficient, smaller applications of fertilizers containing them are required if there is a liberal supply of available nitrogen. The nitrogen can of course be maintained or supplied by the use of legumes in the rotation, by green manure crops, and by the utilization of farm manures and crop residues.

STUDIES ON NONARSENICAL STOMACH-POISON INSECTICIDES[1]

By WILLIAM MOORE and F. L. CAMPBELL, *Fruit Insect Investigations, Bureau of Entomology, United States Department of Agriculture*

NATURE OF THE STUDY

In attempts to find a satisfactory insecticide for the Japanese beetle at Riverton, N. J., a rather hasty survey of possible arsenical substitutes was made, and many compounds too expensive for practical use were tested with the idea of helping to develop useful laws of insect toxicology, a subject exceedingly obscure at present.

PROCEDURE

Since the urgency of the situation would permit no loss of time over unpromising compounds, the method of experimentation chosen does no more than indicate those compounds worthy of more exhaustive tests. All experiments were carried out in small cages; in summer, in the open air under a canvas shelter; in spring and fall, in a greenhouse. A certain number of insects, usually 20, were placed in a cage, and the sprayed or dusted foliage of either potted plants or freshly cut shoots, placed in water, was then introduced. In every case sprayed foliage was allowed to dry before it was placed in a cage. A record was kept of the compound used; its concentration, if used as a spray, in terms of pounds of the insecticide per 50 gallons of water; the number of insects in the cage; the number dead at the end of every 24-hour period after the start of the experiment until it seemed unnecessary to continue it; the percentage killed during seven days, or less, if the test was not run so long; the amount of feeding; and injury to the foliage by the insecticide. In some cases the number of insects that were on the floor of the cage stupefied was also recorded. The extent of feeding was recorded as "slight" if the area eaten was hardly noticeable, "heavy" if the feeding was about the same as that on the control plant, and "medium" if the amount consumed was between the two extremes. In a like manner foliage injury was recorded as "slight" if it could be detected only on close examination, "severe" if all the leaves were practically destroyed, and "medium" if the injury was partial. When the Japanese beetle was not obtainable, tests were made on the tent caterpillar (*Malacosoma americana* Fabricius), the Colorado potato beetle (*Leptinotarsa decemlineata* Say), and the squash lady-beetle (*Epilachna borealis* Fabricius).

DISCUSSION OF EXPERIMENTS AND TABLES

INORGANIC COMPOUNDS

Table I shows the effect of certain relatively insoluble salts on the tent caterpillar. The more soluble compounds were the more injurious to both foliage and caterpillar. Barium carbonate and barium oxalate were tested later on the Japanese beetle (Table II), but were without effect. As a general rule the beetle was more resistant than the other insects to the compounds tested. Antimony, though similar chemically to arsenic, was ineffective in both compounds tested.

[1] Received for publication March 17, 1924.

TABLE I.—*Results, against the tent caterpillar, of spraying wild-cherry foliage with inorganic nonarsenical compounds*

Compound	Rate of application (pounds per 50 gallons water)	Number of insects	First day		Second day		Third day		Fourth day		Fifth day		Sixth day		Seventh day		Per cent killed	Feeding	Foliage injury
			Dropped	Dead	Dropped	Dead	Dropped	Dead	Dropped	Dead	Dropped	Dead	Dropped	Dead	Dropped	Dead			
Barium oxalate	5	74	54	0	53	0	41	11	30	33	22	8	----	----	17	5	77	Medium	Slight.
Barium carbonate.	5	46	39	0	32	0	26	4	17	7	11	16	----	----	9	3	65	do	Do.
Barium fluorid	5	45	12	0	10	0	13	14	4	11	3	0	----	----	5	3	62	Slight	Do.
Barium sulphate.	5	56	1	0	1	0	0	0	----	----	----	----	----	----	----	----	0	Heavy	None.
Barium chromate.	5	46	2	0	2	0	3	0	----	----	----	----	----	----	----	----	0	do	Do.
Barium stearate	5	65	0	0	0	0	8	0	2	0	0	0	0	0	0	0	0	do	Do.
Calcium fluorid	5	139	9	0	3	0	0	0	1	1	0	0	----	----	4	11	9	Slight	Severe.
Calcium oxalate	5	37	0	0	0	0	0	0	0	0	0	0	0	0	----	----	0	Heavy	None.
Lead fluorid	5	46	0	0	1	0	1	0	0	0	0	6	----	----	1	11	37	Slight	Slight.
Lead chromate	5	78	0	0	0	0	0	4	0	0	0	4	----	----	0	1	12	Medium	None.
Zinc fluorid	5	116	4	0	2	0	7	13	9	14	0	22	----	----	0	42	78	Slight	Medium.
Control	----	97	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Heavy	

TABLE II.—*Results, against the Japanese beetle, of spraying or dusting smartweed with inorganic nonarsenical compounds*

Compound	Rate of application (pounds per 50 gallons water)	Number of insects	Number dead							Feeding	Foliage injury
			First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Per cent killed		
Barium oxalate	5	20	0	0	0	2	0	2	0	20	Heavy	None.
Barium carbonate	5	20	0	0	0	0	0	0	----	0	do	Do.
Bismuth carbonate	2	20	0	0	1	0	0	----	----	5	do	Do.
Lead antimonate	2	20	1	0	0	----	----	----	----	5	do	Do.
Do	5	20	0	0	1	0	----	----	----	5	do	Do.
Antimony trisulphid	2	20	0	0	----	----	----	----	----	0	do	Do.
Do	5	20	0	0	----	----	----	----	----	0	do	Slight.
Sodium fluorid	(a)	20	3	11	----	----	----	----	----	70	Slight	Severe.
Control	----	20	0	0	0	0	0	0	0	0	Heavy	

[a] Dusted.

ORGANIC COMPOUNDS

CYANIDS

Considerable work was done on simple and complex metallic cyanids with the dea of finding a cyanid that would be comparatively stable on foliage, and yet would break down in the alimentary canal of the insect with the liberation of deadly hydrocyanic acid.

The so-called lead cyanid is not a simple cyanid of the formula $Pb(CN)_2$, but is a basic salt containing but a small percentage of CN, which varies with the concentrations of the solutions used in its precipitation, and with the extent of its exposure to air and moisture after preparation. Statements in several textbooks in regard to this cyanid conflict. The observations of Williams [3] were confirmed by preparation and analysis of the cyanid in the laboratory. The ineffectiveness of this compound was thought to be due to the low percentage of CN and its rapid volatility in the form of HCN.

[3] WILLIAMS, H. E.—THE CHEMISTRY OF CYANOGEN COMPOUNDS. 423 p. London. 1915.

Zinc cyanid has hitherto been considered a stable cyanid, but under spraying conditions it also loses HCN as the spray dries. This was proved by placing a freshly sprayed plant under a large bell jar closed at the top by a two-holed rubber stopper. Air was aspirated from the jar through two Drechsel wash bottles containing a solution of sodium carbonate to absorb HCN. After air had been drawn through the train for several hours, the contents of the wash bottles were analyzed, and HCN was found in the first bottle, but not in the second. This loss of HCN with large surface exposure of the cyanid to air and moisture was further shown by spraying a mixture of the cyanid and water on a glass plate. After the spray deposit had dried, it was scraped from the glass, weighed, analyzed, and compared with the fresh material. In every case there was a considerable loss of HCN. Finally it was proved that under certain conditions a spray deposit of zinc cyanid would lose all its CN. A plate was sprayed with a mixture of the cyanid and water and the spray deposit was allowed to dry. It was then tested qualitatively for CN. The next day it was sprayed with water to simulate dew, and tested again after it dried. The spraying, drying, and testing were repeated daily till no test was obtained for CN; sufficient evidence for the complete hydrolysis of the compound. Examination of Table III shows that, while zinc cyanid was fairly effective against half-grown caterpillars, it did not kill full-grown caterpillars. It was toxic to the potato beetle (Table IV), but hardly even retarded the feeding of the Japanese beetle (Table V).

TABLE III.—*Results, against the tent caterpillar, of spraying or dusting wild-cherry foliage with cyanids*

Compound	Application		Caterpillars		Number dead							Per cent killed	Feeding	Foliage injury
	Method	Rate (pounds per 50 gallons water)	Number	Stage of growth	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day			
Nickel cyanid	Dust		40	Half-grown	0	3	3	6				30	Slight	None.
Cuprous cyanid	do		40	do	3	13	13	11				100	do	Do.
Zinc cyanid	do		40	do	0	0	0	16				40	do	Do.
Copper nitroprussid.	Spray	10	20	do	0	2	1	1	2	0	5	55	do	Do.
Zinc nitroprusid.	do	10	20	do	0	0	0	0	0	0	0	0	do	Do.
Cuprous cyanid	Dust		20	Full grown	0	0	2	1	1	7		55	do	Do.
Zinc cyanid	do		20	do	0	0	0	1	1	0		10	Medium	Do.
Nickel cyanid	do		20	do	0	0	0	0	0	0		0	Heavy	Do.
Cuprous thiocyanate.	do		20	do	0	0	3	1	1	6		55	Slight	Do.
Cuprous cyanid	Spray	5	20	do	0	0	4	3	1	0	1	45	do	Do.
Zinc cyanid	do	5	20	do	0	0	0	0	0	0	0	0	Heavy	Do.
Nickel cyanid	do	5	20	do	0	0	0	0	0	0	1	5	do	Do.
Cuprous thiocyanate.	do	5	20	do	0	0	10	6	3			95	Medium	Do.
Lead arsenate	do	5	20	do	0	0	2	7	3	4		80	Slight	Do.
Control			20	do	0	0	0	0	0	0		0	Heavy	

TABLE IV.—*Results, against the Colorado potato beetle, of spraying potato and tomato plants with cyanids*

Compound	Application		Number of insects	Number dead							Per cent killed	Feeding	Foliage injury
	Method	Rate (pounds per 50 gallons water)		First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day			
Zinc cyanid	Dust	----	20	3	0	0	1	0	2	----	30	Slight	Yellowing.
Cuprous cyanid	do	----	20	7	2	2	3	0	----	----	70	do	None.
Nickel cyanid	do	----	20	1	0	1	----	----	----	----	10	None	Wilted.
Copper nitroprussid	Spray	10	20	1	2	0	0	1	----	0	20	Slight	None.
Zinc nitroprussid	do	10	20	0	3	0	1	1	----	1	30	do	Do.
Copper nitroprussid	do	5	20	0	0	0	0	0	----	----	0	Medium	Do.
Zinc nitroprussid	do	2	20	0	0	0	0	0	----	----	0	do	Do.
Ammonium nickel cyanid-aniline.	do	10	20	2	1	0	0	1	----	2	30	None	Do.
Ammonium nickel cyanid-phenol.	do	10	20	0	0	0	2	0	0	----	10	Slight	Do.
Ammonium nickel cyanid-benzene.	do	10	20	0	0	0	0	0	0	2	10	do	Do.
Control	----	----	20	0	0	0	0	0	0	0	0	Heavy	

Nickel cyanid, although stable, as shown by long-continued spraying tests similar to the foregoing on glass plates, was not as toxic as zinc cyanid, and, moreover, did not have the physical properties of a good insecticide.

TABLE V.—*Results, against the Japanese beetle, of dusting plants with cyanids*

Compound	Number of insects	Number dead				Per cent killed	Feeding	Foliage injury
		First day	Second day	Third day	Fourth day			
Zinc cyanid	20	0	0	0	----	0	Medium	None.
Cuprous cyanid	20	0	3	1	----	20	None	Do.
Cuprous oxid	20	0	0	----	----	0	Heavy	Do.
Cuprous iodid	20	0	0	0	----	0	Medium	Do.
Cuprous thiocyanate.	20	0	----	----	----	0	Heavy	Do.
Lead thiocyanate	20	0	----	----	----	0	do	Do.
Lead arsenate	20	1	1	2	----	20	Slight	Do.
Control	20	0	0	0	----	0	Heavy	Do.

Cuprous cyanid, CuCN, was tested in the same manner as zinc cyanid and was found to be stable for at least a month, when the glass-plate experiment was discontinued. This cyanid was the only nonarsenical tested which was approximately equal to lead arsenate in toxicity against the Japanese beetle (Table VI). Both the caterpillars and the beetles ate it very sparingly and succumbed quickly. It is hard to wet for a spray mixture. It adheres to foliage excellently as a dust, but is washed off easily by rain. It did not injure the foliage of wild cherry or that of smartweed, but in a special test to determine the point it did injure peach foliage. The writers did not have the opportunity to determine whether or not the burning was due to a trace of the free copper ion.

Since cuprous cyanid was toxic to the Japanese beetle, and nickel cyanid equally stable, was not, it seemed possible that Cu was responsible for the toxicity rather than CN. Therefore other cuprous compounds were prepared and compared with cuprous cyanid. On tent caterpillars cuprous thiocyanate showed remarkable toxicity. It was even more toxic than lead arsenate applied at the same rate. But hopes for its success against the beetle were vain. It

failed completely, as did the other cuprous compounds and another thiocyanate. All efforts to attribute the toxicity of cuprous cyanid to one ion or the other proved fruitless. It is but another of the many instances which show how far we are from understanding the relation between toxicity and the chemical constitution of insecticides.

TABLE VI.—*Results, against the Japanese beetle, of spraying smartweed with cuprous cyanid and lead arsenate*

Compound	Rate of application (pounds per 50 gallons water)	Number of insects	Number dead							Per cent killed	Feeding	Foliage injury
			First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day			
Cuprous cyanid	2	20	0	0	1	3	3	7	----	70	Medium	None.
Do	3	20	1	2	1	0	0	9	----	65	Slight	Do.
Do	4	20	0	0	1	1	1	6	----	45	...do	Do.
Do	5	20	0	2	0	1	2	6	----	55	...do	Do.
Lead arsenate	2	20	0	2	0	1	4	3	----	50	Medium	Do.
Do	3	20	0	2	3	0	4	6	----	75	...do	Do.
Do	4	20	0	0	3	2	5	4	----	70	Slight	Do.
Do	5	20	1	2	2	4	2	2	----	65	...do	Do.
Control	----	20	0	0	0	0	0	0	----	0	Heavy	

The nitroprussids of copper and zinc were prepared and tested. Their toxicity was so low that no effort was made to work out the disposal of CN.

Hofmann and Höchtlen[3] describe additive compounds formed by the addition of benzene, phenol, or aniline to an ammoniacal solution of nickel cyanid. The compounds break down into their component parts on the addition of dilute acid or alkali. With a "shot-gun" remedy of such properties it seemed almost impossible to miss the beetle, but the compounds showed only low toxicity.

ABSORBED ORGANIC COMPOUNDS

It was thought that gaseous, liquid, or soluble solid organic compounds which might be destructive to plant tissues could be applied if they were absorbed on inert materials like clay; it being assumed, of course, that the insect would liberate the adsorbed toxic compound. Unfortunately there was not time to test the assumption by using an adsorbed compound of known toxic properties and of known concentration on the inert material. A series of clays and charcoals was prepared on which such substances as carbazol, anthracene, quiniline, amyl alcohol, and dimethylaniline had presumably been adsorbed. They were entirely without effect; but since the idea was not given a fair trial, it is still worth considering.

A silica gel and a charcoal, on which three war gases and arsenious oxid had been adsorbed, were obtained from the United States War Department. The percentage of each poison in the preparations was given. The gases were not held very tightly at the temperature at which the dusting was done, because their odor was apparent at a distance from the unstoppered bottles. Nevertheless, none of the war gases had the slightest effect on insects as susceptible as the potato beetle and the tent caterpillar (Table VII). Moreover, phosgene, or perhaps its decomposition product HCl, burned the foliage severely. Arsenious oxid burned the foliage as severely as if it had been in water solution.

[3] HOFMANN, K. A., and HÖCHTLEN, F. ABNORME VERBINDUNGEN DES NICKELS. Ber. Deut. Chem. Gesell. 36: 1149-1151. 1903.

TABLE VII.—*Results, against the tent caterpillar and the Colorado potato beetle, of dusting wild-cherry foliage and tomato plants with organic compounds on which war gases had been adsorbed*

TENT CATERPILLAR ON WILD-CHERRY FOLIAGE

Dusts	War gas		Number of insects	Number dead					Per cent killed	Feeding	Foliage injury
				First day	Second day	Third day	Fourth day	Fifth day			
		Per cent									
Silica gel	Phosgene	3.0	20	0	0	0	0	----	0	Heavy	None.
Do	Chloropicrin	20.0	20	0	0	0	0	----	0	...do	Severe.
Do	Chloracetophenone	12.5	20	0	0	0	0	----	0	...do	Slight.
Do	Arsenious oxid	5.0	20	0	12	0	3	----	75	Slight	Severe.
Charcoal	Phosgene	25.0	20	0	0	0	0	----	0	Heavy	Do.
Do	Chloropicrin	35.0	20	0	0	0	0	----	0	...do	None.
Do	Chloracetophenone	11.0	20	0	0	0	0	----	0	...do	Do.
Do	Arsenious oxid	9.0	20	0	11	2	4	----	85	Slight	Severe.
Control			20	0	0	0	0	----	0	Heavy	

COLORADO POTATO BEETLE ON TOMATO PLANTS

		Per cent									
Silica gel	Phosgene	3.0	20	0	0	0	0	----	0	Heavy	None.
Do	Chloropicrin	20.0	20	0	0	0	0	----	0	Medium	Do.
Do	Chloracetophenone	12.5	20	0	0	0	0	----	0	Heavy	Do.
Do	Arsenious oxid	5.0	20	0	----	----	----	----	0	None	Severe.
Charcoal	Phosgene	25.0	20	0	----	----	----	----	0	...do	Do.
Do	Chloropicrin	35.0	20	0	0	0	0	----	0	Heavy	None.
Do	Chloracetophenone	11.0	20	0	0	0	0	----	0	...do	Do.
Do	Arsenious oxid	9.0	20	0	----	----	----	----	0	None	Severe.
Control			20	0	0	0	0	----	0	Heavy	None.

ALKALOIDS

The alkaloids listed in Table VIII were ineffective. The unexpected resistance of insects to these alkaloids has also been observed by Crozier [4] and by Richardson and Smith.[5] The reason for the experiments with alkaloids was detailed in another paper by Moore.[6]

TABLE VIII.—*Results, against the Japanese beetle, of spraying smartweed plants with alkaloids*

Compound	Rate of application (pounds per 50 gallons water)	Number of insects	Number dead						Per cent killed	Feeding	Foliage injury
			First day	Second day	Third day	Fourth day	Fifth day	Sixth day			
Atropine sulphate	0.001	20	0	0	0	0	0	------	0	Heavy	None.
Strychnine sulphate	.002	20	0	0	0	0	0	------	0	...do	Do.
Morphine sulphate	.005	20	0	0	0	0	------	------	0	...do	Do.
Do	.005	20	2	2	0	0	0	------	20	...do	Do.
Veratrin	.005	20	0	0	0	1	0	------	5	...do	Do.
Control	------	20	0	0	0	0	0	------	0	...do	Do.

[4] CROZIER, W. J. REVERSAL OF INHIBITION BY ATROPINE, IN CATERPILLARS. Biol. Bul. 43: 239-245, 1922.

[5] RICHARDSON, C. H., and SMITH, C. R. STUDIES ON CONTACT INSECTICIDES. U. S. Dept. Agr. Bul. 1160, 15 p. 1923.

[6] MOORE, W. THE REACTION OF THE JAPANESE BEETLE TO ARSENICAL SPRAY DEPOSITS. Jour. Econ. Ent. 15: 67-70. 1922.

INSOLUBLE AROMATIC COMPOUNDS

Certain organic compounds insoluble in water can be dusted on foliage without injury to it. Carbazol, acetylsalicylic acid, and acetanilid when so used were quite effective against the Japanese beetle (Table IX), but the season was nearing its close and the lowered resistance of the beetles may have accounted for it. Further tests, however, seemed worth while and were carried out later in the greenhouse on the squash lady beetle (Table X). The effect of various organic compounds on bean foliage is recorded in Table XI.

TABLE IX.—*Results, against the Japanese beetle, of dusting smartweed plants with insoluble aromatic compounds*

Compound	Number of insects	Number dead				Per cent killed	Feeding	Foliage injury
		First day day	Second day	Third day	Fourth day			
Carbazol	20	0	1	7		40	Heavy	None.
Acetanilid	20	0	6	11		85	Slight	Slight.
Acetylsalicylic acid	20	0	12	8		100	do	Do.
Control	20	0	0	2		10	Heavy	

TABLE X.—*Results, against the squash lady beetle, of dusting pumpkin plants with insoluble aromatic compounds*

Compound	Number of insects	First day		Second day		Third day		Fourth day		Fifth day		Sixth day		Seventh day		Per cent killed	Remarks
		Dropped	Dead	Dropped	Dead	Dropped	Dead	Dropped	Dead	Dropped	Dead	Dropped	Dead	Dropped	Dead		
Anthracene	20		0		0		0		0		0		0			0	Beetles were stupefied temporarily.
Anthraquinone	20		0		0		0		0		0		0			0	Plants readily eaten without noticeable effect on beetles.
Tolidin	20		0		0		0		0		0		0			0	Do.
Phenyl-alpha-naphthylamin.[a]	20		0		0		0		0		0		0			0	Beetles were stupefied temporarily.
Acetyl-ortho-amino phenol.	20		0		0	3	0	2	0		2					10	Slow action; no striking effect.
2-6 dichloro-4-nitranilin.	20		1	4	0		0		0		0		0	0	0	5	No noticeable effect.
Diacetyl-ortho-phenylenediamin.	20		0		0		0		0	2	0	4	2		8	50	No striking effect.
4 nitro 2-aminotoluene	20	7	0	16	0	17	0	17	0	18	0	12	0	13	0	0	Beetles were stupefied; 13 finally died.
Ferric-para-nitrobenzoate.	20		0		0		0		0							0	Plants readily eaten without noticeable effect on beetles.
3-nitro-4-aminotoluene.	20	3	0	1	0	4	0	6	0	7	0	7	0			0	Somewhat similar to 4-nitro 2-aminotoluene.
Control	20		0		0		0		0		0		0			0	

[a] Diluted one-half with clay.

TABLE XI.—*Effect of various organic compounds on bean foliage*

COMPOUNDS HAVING THE NH_2 GROUP

Compound	Effect on foliage	Compound	Effect on foliage
Meta-nitroanilin	Medium burning.	Acetyl-para aminophenol	No burning.
2-4-Dichloranilin	Serious burning.	Acetyl-para methylamino-phenol	Do.
2-4-6 Trichloranilin	Medium burning.	Anthranilic acid	Serious burning.
2-6 Dichlor-4-nitroanilin	No burning.	Para-aminobenzoic acid	Medium burning.
Para-chloracetanilid	Serious burning.	Acetyl-para-aminobenzoic acid	No burning.
Para-nitroacetanilid	Do.	Benzamid	Serious burning.
Para-phenylenediamin	Do.	Aminosalicylic acid	No burning.
Meta-tolylenediamin	Do.	Alpha-naphthylamin	Serious burning.
Para-aminoacetanilid	Do.	Beta-naphthylamin	Do.
Diacetyl-ortho-phenylendiamin	No burning.	Phenyl-alpha-naphthylamin	Medium burning.
2- Diaminochlorbenzene	Serious burning.	Nitronaphthylamin	Serious burning.
Para-toluidin	Do.	Diphenylamin	Do.
Acetyl-ortho-toluidin	Do.	Acetyldiphenylamin	Do.
Acetyl-para toluidin	Do.	Carbazol	No burning.
3-Nitro 4-aminotoluene	Do.	Benzidin	Medium burning.
4-Nitro 2 aminotoluene	No burning.	Tolidin	No burning.
Ortho-aminophenol	Serious burning.	Benzanilid	Do.
Para-aminophenol	Medium burning.	Oxanilid	Do.

COMPOUNDS CONTAINING THE COOH GROUP BUT NOT THE NH_2 GROUP

Para-chlorbenzoic acid	Serious burning.	2-4-6 Trinitrobenzoic acid	Serious burning.
Para-nitrobenzoic acid	Do.	5-Nitrosalicylic acid	Do.
2-4-Dinitrobenzoic acid	Do.	Cresotinic acid	Do.
3-5-Dinitrobenzoic acid	Medium burning.	Para-cyanobenzoic acid	No burning.

COMPOUNDS CONTAINING THE OH GROUP BUT NOT THE NH_2 OR COOH GROUP

Para-nitrophenol	Serious burning.	Alpha-naphthol	Serious burning.
Para-nitrophenetol	Do.	Beta-naphthol	Do.
3-5 Nitro-ortho-cresol	Do.		
Trinitro-meta-cresol	Medium burning.		

COMPOUNDS CONTAINING THE NO_2 GROUP BUT NOT THE NH_2, COOH, OR OH GROUPS

Para-nitrochlorobenzene	Serious burning.	Dinitrotoluene	Serious burning.
Para-chlor-nitrobenzene	Do.	Alpha-nitronaphthalene	Do.

COMPOUNDS CONTAINING THE CO GROUP

Anthraquinone	No burning.	Phthalic anhydrid	Serious burning.
Benzil	Do.		

HYDROCARBONS

Diphenyl	Serious burning.	Anthracene	No burning.
Phenanthrene	Do.		

MISCELLANEOUS COMPOUNDS

Hexachlorethane	Serious burning.	Thio-beta-naphthol	Serious burning.
Dichlordiethyl sulphorid	Do.	Triphenyl arsine	Medium burning.

SUMMARY

No inorganic nonarsenical was found of noteworthy toxicity to the Japanese beetle.

The relative stability of simple metallic cyanids was determined in efforts to explain the differences in toxicity among them. When spread out, as in a spray deposit, copper and nickel cyanids are stable, zinc cyanid breaks down slowly, and so-called lead cyanid hydrolyzes quickly.

Copper cyanid was the only nonarsenical tested the toxicity of which was comparable to that of lead arsenate on the Japanese beetle.

Copper thiocyanate showed high toxicity to tent caterpillars, but was nontoxic to the Japanese beetle.

Complex cyanids and other rather complex insoluble organic compounds exhibited low toxicity, and were interesting only from a theoretical point of view.

No definite relations were found between chemical constitution and toxicity.